9th International Congress of the Molecular Biology Association of Turkey

12-14 September 2024 | İzmir Institute of Technology, İzmir, Türkiye

ABSTRACT BOOK





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Dear Members and Colleagues of the Molecular Biology Association,

We are thrilled to announce the 9th International Congress, taking place in İzmir from September 12-14, 2024. This autumn event will be held in conjunction with the EMBO Young Scientists Forum (EYSF), promising an enriching experience for all.

Our host, the İzmir Institute of Technology, will provide a dynamic scientific program with esteemed speakers. The congress will be a hub for knowledge exchange and collaborative discourse, promoting molecular biology's progress.

The EYSF, on September 10-11, will precede the congress, offering emerging scientists a stage to interact with experts and present their innovative research.

For the latest updates and announcements, please follow our official congress website, social media channels, and subscribe to our newsletter.

We warmly invite you to join us in the beautiful setting/surroundings of Urla, İzmir, for an inspiring exchange of ideas and insights. Your participation is greatly anticipated.

Sincerely,

Prof. Dr. Batu Erman
President of the Molecular Biology Association

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ABSTRACTS
INVITED SPEAKER

P01
Control of Cellular Proteostasis by the Ubiquitin-selective Unfoldase p97/VCP

Alexander Buchberger

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Ubiquitylation, the covalent attachment of the small protein ubiquitin to target proteins, regulates numerous cellular processes in all eukaryotes. In particular, ubiquitylation controls protein degradation through three key proteolytic systems: the 26S proteasome, macroautophagy, and the endolysosomal pathway. The chaperone-related, ubiquitin-selective ATPase p97 (also known as VCP in mammals and Cdc48 in plants and lower eukaryotes) plays a central role in many ubiquitin-dependent processes. With the help of dedicated cofactors, p97 recognizes ubiquitylated substrate proteins and utilizes ATP hydrolysis to unfold them, directing them towards degradation or alternative cellular fates. Its critical involvement in these pathways makes p97 a key regulator of eukaryotic protein homeostasis ('proteostasis'). Importantly, missense mutations in the human VCP gene encoding p97 cause multisystem proteinopathy 1 (MSP1), a heterogeneous disorder that includes inclusion body myopathy, frontotemporal dementia, Paget's disease of the bone, and amyotrophic lateral sclerosis (ALS). On the cellular level, these conditions are characterized by cytoplasmic protein inclusions (aggregates) that are positive for ubiquitin and TDP-43, a protein commonly associated with neurodegenerative diseases, strongly suggesting that the failure of mutant p97 to prevent the formation and/or to promote clearance of protein aggregates contributes to the pathogenesis of MSP1. In this presentation, I will discuss our lab's recent findings on the role of p97 in clearing two specific subcellular structures formed under proteotoxic stress: stress granules and aggresomes.

Stress granules (SGs) are cytoplasmic clusters of messenger ribonucleoproteins (mRNPs) that are stalled during translation initiation. These granules form in response to various stress conditions, including exposure to the environmental toxin and carcinogen arsenic. While impaired SG turnover has been linked to neurodegenerative diseases, the precise mechanisms governing this process remain unclear. Our research identified the p97 cofactor ZFAND1 as an evolutionarily conserved regulator of SG clearance. ZFAND1 interacts with p97 and the 26S proteasome and recruits them to arsenite-induced SGs. Upon depletion of ZFAND1, SGs lacking p97 and the proteasome accumulate defective ribosomal products and persist after arsenite removal, indicating their transformation into aberrant, disease-associated SGs.

Aggresomes are large, perinuclear structures that sequester protein aggregates when cellular protein quality control (PQC) systems are overwhelmed. Both the formation and clearance of aggresomes are critical for maintaining proteostasis, and defects in these processes have been implicated in neurodegenerative diseases. Our findings reveal that heterogeneous, amorphous aggresomes containing endogenous proteins are cleared via selective autophagy ('aggrephagy') requiring the cargo receptor TAX1BP1. Through TAX1BP1 proximitome analysis, we identified various PQC factors associated with aggresomes, including Hsp70 chaperones, the 26S proteasome, and p97. Hsp70 and p97 with its cofactors UFD1-NPL4 and FAF1 are essential for aggresome disassembly, while the 26S proteasome is not required. Substrate proteins within aggresomes are degraded via different routes, in part in a p97-dependent manner via aggrephagy. Inhibition of p97 prevents the disintegration of aggresomes and their incorporation into autophagosomes, despite the presence of factors required for autophagosome formation. Thus, the p97-mediated removal of ubiquitylated aggresomal substrate proteins is essential for the disintegration and subsequent piecemeal autophagy of aggresomes.

Keywords: p97VCP, Cellular Proteostasis, Proteolytic Systems

P02

Engineering Pancreas Organoids to Study Pancreas Development and Disease

Anne Grapin-botton

Max Planck Institute of Molecular Cell Biology and Genetics

To attempt to understand human pancreas development, as a complement to investigations in mice and with mouse pancreas organoids, we established 3D (three-dimensional) culture conditions that enable the efficient expansion, differentiation and morphogenesis of pancreatic progenitors isolated from human fetuses or produced from human pluripotent stem cells (hPSCs). Our 3D culture conditions enable the efficient expansion, differentiation and morphogenesis of mouse and human pancreatic progenitors isolated from fetuses or produced from human pluripotent stem cells (hPSCs). Using single-cell sequencing, we compared the transcriptional profile of cells grown in vitro in 2D, and 3D from hPSC-derived, to those isolated from fetal pancreas. Our experiments show a good molecular stability of pancreas progenitors over time, retention of the endocrine differentiation capacity and a molecular signature closer to the in vivo counterparts when grown in 3D than in 2D. We used these systems to study differentiation dynamics towards the endocrine lineage. Miniaturizing the system to 384-well plates we performed a high content imaging screen for small molecules affecting organoid cell composition and morphogenesis. We developed an image analysis pipeline and identified compounds affecting lumen size and shape, pancreas progenitor identity as well as expansion. The human organoids also open a way to investigate whether some genes identified in genome-wide association studies (GWAS) control the development of beta cells thereby predisposing to diabetes later in life.

Keywords: Pancreas Organoids, Engineering, Pancreas Development

P03

Structural Characterization of IP3 Receptor Calcium Channels by cryo-EM

Erkan Karakaş

Vanderbilt University School of Medicine

Calcium ions (Ca2+) serve as a critical signaling molecule, regulating a myriad of cellular functions in response to various stimuli. Central to the Ca2+ signaling paradigm are the inositol 1,4,5-triphosphate (IP3) receptors, which are intracellular Ca2+ channels predominantly found in the endoplasmic reticulum (ER) membrane. These channels are activated by IP3, which is produced following the activation of G-protein coupled receptors. The activation of IP3 receptors facilitates a rapid Ca2+ release from the ER into the cytoplasm, initiating a variety of signaling pathways that govern essential physiological processes, including learning, fertilization, gene regulation, and programmed cell death. Malfunctions in IP3 receptor function can lead to aberrant Ca2+ signaling, contributing to the pathogenesis of diseases such as diabetes, cancer, and neurodegenerative conditions. IP3Rs are activated by the binding of IP3 and Ca2+, with their activity being inhibited by elevated Ca2+ levels and enhanced by ATP. Despite their significance, the precise molecular mechanisms governing their activation and regulation have remained elusive, primarily due to the absence of active conformational structures. In this presentation, we will explore cryo-electron microscopy (cryo-EM) structures of the human type-3 IP3 receptor in various gating states, including IP3-ATP bound pre-active states with closed channels, an IP3-ATP- Ca2+ bound active state with an open channel, and an IP3-ATP- Ca2+ bound inactive state with a closed channel. These structures reveal the conformational dynamics initiated by IP3 binding that prepare the receptor for activation by Ca2+, the subsequent channel opening triggered by Ca2+ binding, the modulation by ATP, and the dilation of the channel pore. This seminar will illuminate the complex molecular interactions and structural transitions that govern the activation and regulation of IP3 receptors. By providing a detailed view of these processes, we lay the groundwork for advancing our understanding of IP3 receptor physiology and pathology, paving the way for innovative research into their biophysical properties and roles in cellular signaling. Moreover, we will discuss the methodology behind cryo-EM, emphasizing its invaluable contribution to our understanding of complex molecular mechanisms and its transformative impact on the field of structural biology.

Keywords: IP3, Receptor Calcium Channels, cyro-EM

P04

Exploring the Cancer Transcriptome with k-mers

Daniel Gautheret

Université Paris-saclay

The transcriptome is where genetic, epigenetic and environmental signals converge to produce functional RNA molecules. Modern transcriptomic data (RNA-seq) should thus offer a unique view into the inner workings of the cell. However, the way RNA-seq is usually interpreted falls short of providing this exhaustive picture. RNA-seq data is usually converted into a gene expression table using reference genes. That is to say, all non-canonical RNAs produced by genetic and epigenetic variation are not reported. Here we argue that the study of human cancer would hugely benefit from methods capable of analyzing RNA-seq data in a de novo fashion. In a recent application of these methods, we used data from baseline melanoma samples and sought RNAs associated to immunotherapy response. We identified unannotated RNA sequences expressed from intergenic and centromeric regions in a subset of patients who do not respond well to immunotherapy. These sequences are among the rare biomarkers of immunotherapy resistance. To conclude my talk, I will present current efforts to index very large cancer datasets (>100,000 samples) in pursuit of the holy grail: identifying all cancer-specific RNAs.

Keywords: Transcriptome, k-dimers, RNA

P05
RNA Processing Across Evolution

Tuğçe Aktaş

Max Planck Institute For Molecular Genetics

RNA splicing is a eukaryotic innovation and even though the spliceosome machinery is mostly conserved from yeast to humans, the number, length and distribution of introns is highly variable and can rapidly change during evolution. One of the contributing factors to this diversity is the expansion of transposable elements (TEs) that make up 47% of human introns. During mRNA synthesis, intronic TEs are transcribed along with their host genes but rarely contribute to the final mRNA product because they are spliced out and degraded. Interestingly, TEs are an abundant source of RNA-processing signals through which they can create new introns and chimeric transcripts. We work on several aspects of transposon-host interactions and their impact on the evolution and wiring of post-transcriptional RNA processing networks. We take candidate-approach to study RNA binding proteins (RBPs) that are implicated in exon and intron selection for a mechanistic understanding of the exclusion of TEs from the final mRNA. In addition, we take unbiased approaches and study RBP hubs, such as Nuclear Speckles, to investigate how strategies that suppress, delay or neutralize transcribed transposons/viruses shape our transcriptomes and through evolution, our genomes. Results from our most recent work will be presented.

Keywords: RNA, Evolution, Eukaryotic Innovation

P06

tRNA-Derived RNAs: Biogenesis, Numbers, Molecular Interactions and Functional Potential of a Cellular 'Waste Product'

Matthias Schaefer

Medical University of Vienna

Stress-induced tRNA fragmentation is an evolutionary conserved process that is catalyzed by endonucleolytic activities targeting mature tRNAs. The resulting tRNA-derived RNAs (tDRs) are detectable in many biological systems, and experimental manipulation indicated that these small RNAs could affect various biological processes. How highly structured tRNAs, containing only one hydrolytic "nick" in their sequence, give rise to individual and potentially biologically active tDRs remains poorly understood. Furthermore, the mode of action of individual tDRs at physiological copy numbers, that is molecules produced per cell, as interaction partners with other molecules, has not been determined.
How many specific tDRs are produced per cell, where do they localize, especially during the stress response, and which proteins do they interact with? Our lab addresses these questions using biochemical and imaging methodology with a keen eye on quantifying molecular numbers, which form the basis for forming and testing specific hypotheses as to the function of these small RNAs. In my talk, I will discuss the current state of research, point out numerous misconceptions in the tDR biology field, and describe our progress in better understanding the functional potential of tDRs.

Keywords: Biogenesis, Stress-induced tRNA, Waste Product

P07

Biorad Droplet Digital PCR Technology and Its Most Recent Applications in Molecular Biology

Letizia Ciccone

P08

The Art of Making Multifunctional Antibodies for Cancer Therapy

Harald Kolmar

Technische Universität Darmstadt

Over the last few decades, monoclonal antibodies have become an invaluable class of therapeutics for the treatment of various diseases. Below high affinity and specificity, low immunogenicity and high-level expression, biophysical properties such as low aggregation behaviour, high solubility, covalent stability of disulfide bonds etc. are key aspects that have to be taken into consideration in the antibody discovery process. In recent years, rather complex antibody formats such as bispecific and multispecific antibodies emerged posing even higher demands on their developability. We recently established a platform technology for the generation of multispecific antibodies with beneficial functional and biophysical properties starting from immunization of chickens. Examples for the discovery of such type of molecules will be presented for potential applications in cancer therapy.

Keywords: Cancer, Antibodies, Immunization

P09
A DNA Toolbox for Spatial Biology from Imaging to Sequencing

Sinem Saka

European Molecular Biology Laboratory

DNA is not only a fundamental constituent of cells, but has also great capacity for information storage. We leverage the predictability of DNA hybridization kinetics and orthogonality of DNA sequences to utilize DNA oligos as tagging and barcoding tools for improving the major limitations of in situ visualization of molecules. We have previously developed multiplexed imaging approaches such as SABER-FISH and Immuno-SABER that utilize DNA barcoding and a flexible in situ signal amplification system for efficient visualisation of many protein, DNA or RNA targets in cells and tissues. More recently, we have implemented DNA barcoding for a new spatial transcriptomics approach, Light-Seq, which directly integrates fluorescence imaging and whole-transcriptome next-generation sequencing of the same cells in fixed biological samples. Light-Seq combines spatially-targeted, rapid photocrosslinking of DNA barcodes onto cDNAs in situ with a novel one-step DNA stitching reaction to create pooled, spatially-indexed sequencing libraries. This light-directed barcoding enables imaging-based in situ selection of multiple cell populations in intact fixed tissue samples for full transcriptome sequencing based on location, morphology, or protein stains, without cellular dissociation. Applying Light-Seq to mouse retinal sections, we discovered new biomarkers for a very rare neuronal subtype, dopaminergic amacrine cells, from only 4-8 individual cells per section. Light-Seq provides an accessible workflow to combine in situ imaging and protein staining with next-generation sequencing of the same cells, leaving the sample intact for further analysis post-sequencing. We are currently using these methods to address the fundamental question of how single-cell identity relates to spatial and molecular organisation. By co-investigating different facets of multi-dimensional cellular phenotypes, we aim to contribute to uncovering the complexity of intricate diseases like cancers and neurodegeneration and provide new insights into cellular homeostasis and disease formation.

Keywords: DNA, Toolbox, Spatial Biology

P10
Navigating the Aging and Neurodegenerative Brain

Özgün Gökçe

German Center For Neurodegenerative Diseases (dzne), Medical Faculty, University of Bonn

Recent advancements in single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics (ST) have paved the way for a deeper understanding of the cellular mechanisms underlying the aging process. A key feature of nervous system aging is the decline in white matter volume and function; however, the specific mechanisms driving white matter pathology remain elusive. Through the application of spatial transcriptomics and scRNA-seq, we identified age-associated alterations in the identities of microglia and oligodendrocytes within white matter. To further characterize these cellular responses, we developed a novel methodology termed Spatial Transcriptomics-correlated Electron Microscopy (STcEM), which integrates large-area scanning electron microscopy (EM) with multiplexed error-robust fluorescence in situ hybridization (MERFISH). This technique enables the correlation of transcriptional identities of single cells with their corresponding ultrastructural data. Our findings highlight the important role of CD8+ T cell-induced interferon-responsive oligodendrocytes and microglia as modulators of white matter aging and contributors to neurodegenerative disorders.

Keywords: Neurodegenerative Brain, Spatial Transcriptomics, scRNA-seq

P11

What Doesn't Kill You Makes You Stranger: Alternative Outcomes of Cell Death signals

Seamus J. Martin

Trinity College Dublin

Cancer chemotherapeutic agents are utilized to kill transformed cells, but most cells fail to die upon a single exposure of such agents in a therapeutic setting. Over the past two decades, much effort has been directed towards understanding the molecules which dictate whether cells live or die upon exposure to cytotoxic stimuli, and we now have an impressively detailed understanding of the cell death machinery. However, a frequent outcome of exposure of cells to potentially lethal doses of chemotherapeutic agents is the initiation of premature replicative senescence, a state where cells fail to die but exit the cell cycle and acquire several unusual characteristics including the secretion of multiple pro-inflammatory cytokines. Senescent cells can be detrimental to tumor clearance due to their pro-inflammatory properties and have been implicated in eliciting a tumor-protective immune response. For the foregoing reasons, there is much interest in how cells evade chemotherapy and enter senescence as well as strategies to eliminate senescent cells. Here, I will discuss the interplay between the cell death and senescence machinery and present data to suggest that 'death receptors' as well as their downstream target, caspase-8, play key roles in policing entry to DNA damage-induced senescence.

Keywords: Cells, Chemotheraputic Agents, Death Signals

P12

Biological Engineering of Biomolecules in Mammalian Cells

Mutlu Erdoğan

Ankara University

Biomolecules engineered through directed evolution are used to manufacture many things from pharmaceuticals to biofuels. Moreover, protein- and RNA-based “living drugs” developed using biological engineering methods can be employed in therapeutics. However, recent directed evolution methods apply isolated biomolecule engineering schemes in tube without cell context. Yet, for engineering of many biomolecule types, the proper context for phenotypic selection is mammalian or human cells, but generating biomolecule libraries in mammalian cells still faces numerous challenges. Here I present strategies for massive diversification of proteins via CRISPR-Cas systems, which allows producing panels of mammalian cells expressing heterologous and complex sequence libraries. To demonstrate the utility of the method, an extremely pH-resistant red fluorescent protein variant suitable for labeling of acidic organelles is engineered in situ in lysosomes via an approach based on CRISPR/Cas9- mediated protein diversification. These methods enable engineering of proteins for which the mammalian cell context or organelle localization is necessary, and facilitate tailoring their properties under precise conditions of sub-cellular compartments for functionality optimization.

Keywords: ER, Tumor Microenvironment, Cancer Immunotherapy

P13

Interplay of ER stress signaling and the tumor microenvironment – Implications for cancer immunotherapy

Fahri Saatçioğlu

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Endoplasmic reticulum (ER) is the largest organelle in the cell. Among others, it functions as a stress sensor responding to increased demand for protein production and folding under both physiological and pathological conditions. ER stress initiates a series of coping mechanisms that are termed the unfolded protein response (UPR), which restructures the cellular transcriptional, translational, and degradation pathways to help resolve the defects in protein folding. ER stress is common in cancer cells due to increased protein synthesis necessary for their deregulated proliferation, chromosomal abnormalities affecting membrane and secreted protein production, limited nutrient availability, and low oxygen tension; thus, UPR is often hijacked by tumor cells for their survival. However, how activation of UPR in cancer cells may affect the tumor microenvironment (TME) remain largely unexplored.
We investigated the potential role of IRE1α-XBP1s signaling, one of three canonical UPR pathways, on modulation of TME dynamics in prostate cancer (PCa). We found that IRE1α is increased in PCa patients; consistently, genetic or pharmacological inhibition of IRE1α-XBP1s signaling in multiple syngeneic or orthotopic mouse PCa models dramatically reduced tumor growth. Multiomics analysis of tumor samples upon IRE1α deletion in cancer cells showed significantly potentiated interferon (IFN) response and activation of immune system related pathways in the TME. Single-cell RNA sequencing (scRNA-seq) analysis revealed that the abundance of immunosuppressive cells, such as tumor-associated macrophages (TAMs), pericytes, cancer-associated fibroblasts, and T regulatory cells were markedly reduced in the IRE1α deficient tumors. Analysis of differentially expressed genes in various cell types in the TME demonstrated that expression signatures associated with IFN responses were significantly enriched in TAMs, cancer cells, and dendritic cells. In addition, a novel scRNA-seq derived TAM gene signature is strongly associated with poor PCa survival. Importantly, IRE1α inhibition by the small molecule MKC8866 (ORIN1001) that is in clinical trials significantly enhanced anti-PD-1 checkpoint inhibitor therapy in syngeneic mouse PCa models. Our findings indicate that IRE1α not only promotes cancer cell growth and survival, but it also strongly inhibits anti-tumor immunity in the PCa TME.

Keywords: Biomolecules, Mammalian Cells, Biological Engineering

P14

Novel Mechanisms and Therapeutic Targets in Skeletal Muscle Atrophy

Serkan Kır

Koç University

Skeletal muscle atrophy is a hallmark of the cachexia syndrome that is associated with poor survival and reduced quality of life in cancer patients. Muscle atrophy involves excessive protein catabolism and loss of muscle mass and strength. Inflammatory cytokines have been implicated in muscle atrophy, however, available anti-cytokine therapies fail to prevent muscle wasting in cancer patients. An effective therapy against muscle wasting is lacking as mechanisms driving the atrophy process remain incompletely understood. Our recent work has revealed the upregulation of Ectodysplasin A2 Receptor (EDA2R) and Oncostatin M Receptor (OSMR) in tumor-bearing mice and cachectic cancer patients. Activation of EDA2R and OSMR signaling pathways promotes skeletal muscle atrophy. Stimulation of primary myotubes with EDA2R ligand, EDA-A2, or OSMR ligand, OSM, triggers pronounced cellular atrophy via inducing the expression of muscle atrophy-related genes, such as Atrogin1 and MuRF1. While EDA-A2 drives myotube atrophy via activating NFκB-inducing Kinase (NIK) and the noncanonical NFκB pathway, OSM triggers this process utilizing the JAK/STAT3 pathway. Both EDA-A2 and OSM promote muscle wasting in mice when overexpressed in this tissue. Tumor-bearing mice lacking EDA2R or muscle NIK are resistant to muscle wasting. Tumor-induced OSM upregulates muscle EDA2R expression and muscle-specific depletion of OSMR or neutralization of the circulating OSM preserves muscle mass and function in tumor-bearing mice. Our results demonstrate that EDA2R/NIK signaling mediates cancer-associated muscle atrophy in an OSM/OSMR-dependent manner. Thus, therapeutic targeting of these pathways may be beneficial in preventing muscle loss.

Keywords: Novel Mechanisms, Therapeutic Targets, Skeletal Muscle Atrophy

P15

Harnessing Synthetic Lethality in Breast Cancer Therapy

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[3] Vancouver Prostate Centre, University of British Columbia

Breast cancer, characterized by its heterogeneous structure, exhibits morphological and molecular differences within tumor masses. This variability often limits the effectiveness of treatment methods across all patients. Breast cancer is classified into subtypes: luminal A, luminal B, HER2-positive, and triple-negative breast cancer (TNBC). The progression of the disease and response to treatment vary depending on these subtypes. Particularly, ER-positive breast cancer expresses estrogen receptors (ER), and treatments typically target the ERα signaling pathway. However, some patients exhibit resistance to endocrine therapy, making it crucial to understand the mechanisms underlying this resistance to develop effective treatment strategies. Mutations in the ARID1A gene play a significant role in cases of endocrine-resistant breast cancer. These mutations disrupt the function of the SWI/SNF complex and lead to synthetic lethal interactions in the absence of other tumor suppressor genes. Thus, identifying ARID1A and its synthetic lethal partners is a critical step in advancing personalized medicine. The aim of this project is to discover new synthetic lethal epigenetic partners in ARID1A-mutated luminal breast cancer cells. Systematically identifying these partners has the potential to enhance endocrine response in luminal breast cancer patients and identify new drug targets. Through our CRISPR genetic screening, we have identified four epigenetic factors that may have a synthetic lethal interaction with ARID1A. This project aims to validate these factors and elucidate their mechanisms of action using molecular techniques. The results will provide new insights into personalized medicine for cancer treatment.

Keywords: Synthetic Lethality, Breast Cancer Therapy, Endocrine-resistant

P16
RareBoost ERAChair Project

Uğur Özbek

P17

The Fine Art of Nanoplate-Based Digital PCR: A Cutting-Edge Transformation for Molecular Biology Applications

Onur Bender (Satellite Symposium)

P18

Galectin-3 AND 9 Bind to TRAIL Agonist Receptors to Regulate TRAIL-induced Apoptosis

Olivier Jean-pierre Micheau

Inserm

APO2L/TRAIL (TNF-related apoptosis-inducing ligand) induces apoptosis in tumor cells by engaging the transmembrane glycoproteins DR4 and DR5, which are modified with N- and O-glycosylations, respectively. Although chemotherapy is known to enhance TRAIL-induced apoptosis, the precise molecular mechanisms behind this effect remain unclear. Here, we provide evidence that chemotherapeutic agents like 5-FU sensitize tumor cells to TRAIL-induced apoptosis through the action of lectins. We demonstrate that galectin-9, along with galectin-3, directly interacts with TRAIL receptors, contributing to apoptosis similarly to conventional DISC (Death-Inducing Signaling Complex) components. Specifically, galectin-9 binds to the extracellular domains of DR4 and DR5, with this interaction being dependent on the receptors' glycosylation status. Overexpression or exogenous addition of recombinant galectin-3 or galectin-9 alone significantly increases TRAIL sensitivity. Furthermore, the loss of glycosylation in DR4 or DR5—achieved through site-directed mutagenesis or production in prokaryotic cells—impairs galectin-9 binding. Altogether, our results identify galectins as novel components of the TRAIL DISC, offering a potential explanation for the synergistic effects of chemotherapeutic drugs with TRAIL.

Keywords: Galectin-3, TRAIL, Apoptosis

P19

Physics of Cells and Nanoscale Bioparticles as a New Biomarker for Health and Disease

Erdinç Sezgin

Science For Life Laboratory, Department of Women

Remodelling of our cells as response to environmental changes is essential for their survival and function. Ability of immune cells to pass through tight epithelial cell layers from circulating blood during infection, ability of tumour cells to travel through the body during metastasis, migration potential of the cells after epithelial-to-mesenchymal transition could be examples where cells undergo extensive remodelling. Although numerous studies aimed at finding protein markers during such cellular processes, there is a major gap in our understanding of how collective biophysical properties of the cells (such as stiffness, membrane fluidity, viscosity etc) alter during these crucial biological processes. Similarly, our understanding of how biophysical properties of cells change in diseases is also limited. To gain a thorough mechanistic perception of cellular processes and diseases, it is essential to fill this gap and have a clear and quantitative picture of biophysical remodelling of the cells.

We and others have made extensive effort to unravel the biophysical aspects of cells in a quantitative manner. To achieve this, we developed advanced imaging approaches that could reveal the molecular details with very high spatiotemporal resolution. These technologies allowed us to see how biophysical properties of cells play crucial roles for signalling from molecular to cellular level. Although these technologies were extremely useful to study biophysical aspects of cellular life at the molecular level, their low sampling (one cell at a time) has been a major obstacle to apply them to medical problems that require measuring thousands of cells. This can be overcome with high throughput methodologies that can robustly report on the ensemble biophysical properties of cells which require reliable reporters and instruments. Thus, while developing advanced instrumentation, we also develop reliable probes to quantify different biophysical properties of cells. Here, I will discuss our approach from probe development to high throughput biophysical analysis.

Keywords: Nanoscale Bioparticles, Physics of Cells, Pellular Processes

P20

Unlocking the Potential TCR-NK Cells for Cancer Immunotherapy

Tolga Sütlü

Acıbadem Mehmet Aydınlar Üniversitesi

Antigen-specific retargeting of cytotoxic lymphocytes against tumor-associated antigens has so far remained largely dependent on chimeric antigen receptors (CARs) that can be constructed by the fusion of an extracellular targeting domain (classically a single-chain variable fragment from an antibody) fused with intracellular signaling domains to trigger activation of T or natural killer (NK) cells. A major limitation of CAR-based therapies is that this technology only allows for the targeting of antigens that would be located on the surface of target cells while non-surface antigens, which affect approximately three-fourths of all human genes, remain out of reach. The targeting of non-surface antigens is only possible using inherent T cell receptor (TCR) mechanisms. The clinical development of genetically modified TCR-T cells has so far been hampered by problems due to the mispairing of endogenously produced TCR chains with the ectopically delivered ones. Since NK cells do not express any endogenous TCR chains, TCR modification of NK cells stands out as a potential solution to this problem, enabling the antigen-specific targeting of intracellular proteins. Our group has previously shown the proof-of-concept for NK cells genetically modified to express functional T-Cell Receptors (TCR-NK cells). NK cells expressing CD3δ, CD3γ, CD3ε and TCRα/ß on their cell surface can detect an antigenic peptide presented by MHC-I and selectively show antigen-specific cytotoxic activity. The overexpression of CD3ζ in TCR-NK cells does not seem to increase antigen-specific triggering but on the contrary, shows increased background activity against non-specific targets. However, the underlying mechanisms of increased non-specific activity caused by the addition of CD3ζ is unknown.

Keywords: TCR-NK Cells, Cancer, Immunotherapy

P21

Exploring the Tumor Immune Microenvironment and Markers of Immunotherapy Response Using a Novel Visual Data Analysis Interface

Atakan Ekiz

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Analyzing the cancer transcriptomics data in the TCGA and other public repositories requires coding skills that are associated with a steep learning curve for most non-specialists. To enable a wider utilization of these data, we introduce The Cancer Genome Explorer (TCGEx), a web-based R/Shiny visual interface that can perform a number of sophisticated analyses ranging from survival modeling and gene set enrichment analysis to unsupervised clustering and linear regression-based machine learning. In addition to providing access to preprocessed data from TCGA and immune checkpoint inhibition studies on cBioportal and CRI-iAtlas, the TCGEx platform allows users to upload and investigate their own data. Using this tool, we investigated human cutaneous melanoma and identified sex-dependent survival differences as well as gene expression patterns in mutational subsets. Our analyses revealed the heterogeneity within the tumor immune microenvironment and a positive relationship between T cell receptor diversity and interferon gamma (IFNg) expression within the tumor. IFNg signaling is critical for antitumor immunity and microRNAs (miRNAs) play a key role in regulating this pathway. We utilized the lasso machine learning algorithm featured in TCGEx to identify miRNAs that are most closely related to IFNg pathway activation. This approach uncovered miR-155 as one of the key miRNAs associated with intratumoral immune activation and improved survival. We validated these findings in melanoma biopsies prior to treatment using data from multiple immune checkpoint inhibition (ICI) trials. Although miR-155 expression was increased in some ICI-responsive tumor samples, this finding was not consistent across other data sets. To broadly identify transcriptomic markers associated with immunotherapy response, we integrated data from multiple studies and applied lasso regression to binarized therapy outcomes, focusing on predictors selected from genes encoding cytokines and their receptors. Our findings identified a set of 5 cytokines (IFNg, CXCL9, CXCL13, CCL5, and IL-21) whose expression distinguishes responders from non-responders across different cancer and treatment types. Taken together, the TCGEx platform aids in extracting meaningful insights from cancer transcriptomics data through user-friendly visual interfaces. Freely available at https://tcgex.iyte.edu.tr, TCGEx pipelines can also be adapted to other study contexts to investigate mechanisms of human cancer.

Keywords: Microenviroment, Immunotherapy Response, Data Analysis Interface

P22
Single-cell Driven Enhancer Modelling and Design

Stein Aerts

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The combination of scRNA-seq and scATAC-seq allows building gene-regulatory atlases of any tissue and species. I will present several new computational strategies that exploit single-cell multi-omics data: (1) to model genomic enhancers using topic modelling and convolutional neural networks; and (2) to derive "enhancer-GRNs" (eGRN) with key transcription factors, genomic enhancers, and predicted target genes per cell type. I will discuss the results of several case studies where we applied these strategies, including the Drosophila brain, human melanoma, the mouse liver, and the evolution of cell types in the vertebrate telencephalon. Finally, I will discuss how enhancer models based on deep learning can be exploited to design synthetic enhancers for Drosophila and human cell types.

Keywords: scRNA-seq, scATAC-seq, eGRN

P23

SUMOylation in Health and Disease and its Potential for Targeted Therapies

Umut Şahin

Boğaziçi University

My lab studies the biology of SUMO (Small ubiquitin-like modifier) proteins, their role in pathogenesis, and their potential as drug targets. SUMOylation is an evolutionarily conserved post-translational protein modification essential for normal cell function and is disrupted in multiple disease states, including cancer, neurodegeneration, and infectious diseases.

We conduct curiosity-driven fundamental research to dissect the functions of SUMOylation in vital cellular processes, disease biology, drug discovery and genome engineering.

My group is particularly interested in understanding the cellular mechanisms leading to neurodegeneration in ALS (Amyotrophic Lateral Sclerosis), a neurodegenerative disease that represents a critical unmet medical need. We use multidisciplinary approaches including biochemistry, biophysics, proteomics, genetics and transgenic mouse models to explore the potential of SUMOylation as a target process that can be manipulated with therapeutic benefits in ALS. Here, I will discuss our recent efforts to develop and validate a SUMO modulating targeted therapy that can now diminish disease incidence, alleviate symptoms and extend lifespan in a preclinical model of ALS. Our results demonstrate the feasibility of SUMO-mediated protein degradation as a druggable node to treat neurodegenerative diseases and could have direct consequence in patient care.

Keywords: SUMO, ALS, Cell

P24

Nur Mustafaoğlu

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ABSTRACTS
SHORT TALKS

ST01

Proximity labeling and SILAC based proteomic approach identifies proteins at the interface of homotypic and heterotypic cancer cell interactions.

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Background/aim: Cell-cell interactions are critical for the growth of organisms and maintaining homeostasis. In the tumor microenvironment, different types of cell interactions promote cancer progression. Given their importance in healthy and diseased conditions, we developed a method combining proximity labelling and quantitative proteomics to profile the cell-cell interactome using co-culture system.

Materials and methods: This study employed a combinatorial approach of horseradish peroxidase (HRP)-dependent proximity biotinylation and stable isotope labeling by amino acids in cell culture (SILAC) to identify proteins involved in homotypic (same cell type) and heterotypic (different cell types) cancer cell interactions. Epithelial (MCF7) and mesenchymal (MDA-MB-231) breast cancer cells were co-cultured to model these interactions. HRP was targeted to the extracellular leaflet of the plasma membrane in 'bait' cells to label proximal proteins in 'prey' cells. Prey cells were metabolically labeled with heavy isotopes, enabling differentiation of self-biotinylated proteins of bait cells. Biotinylated proteins were affinity purified and analysed with mass spectrometry. To test the efficiency of proximity labelling in spheroids, cells were encapsulated in alginate hydrogel beads via CaCl2-based ionic crosslinking.

Results: We mapped both the homotypic and heterotypic interactomes of epithelial and mesenchymal breast cancer cells. The enrichment of cell surface and extracellular proteins confirms the specificity of our methodology. This method revealed prominent signaling pathways orchestrating homotypic and heterotypic interactions of epithelial and mesenchymal cells. It also highlights the importance of exosomes in these interactions. The efficient proximity biotinylation in spheroids suggested that the methodology can be extended to 3D co-culture models.

Conclusion: This study presents a new pipeline of experimental protocols and strategies, along with bioinformatic analyses, that can be used to comprehensively examine the proteome of any type of cell-cell interaction.

Keywords: Cell-cell interaction, proximity labeling, SILAC, proteomics, signaling pathways

ST02
Identification of AXL as a transactivation target of p73

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Background/Aim: AXL is a critical receptor tyrosine kinase implicated in drug resistance across various cancers. Elevated AXL overexpression is frequently observed in tumors that become resistant to chemotherapy, radiotherapy, and targeted therapies. The mechanisms underlying the upregulation of AXL in cancer cells remain poorly understood.Our previous research indicated that a variant of p73 can induce AXL expression in hepatocellular carcinoma (HCC) cells.Furthermore, a p73 binding motif was identified in the promoter region of AXL. This led us to hypothesize that p73 may act as a transcription factor responsible for AXL upregulation in cancer cells.

Materials and Methods: We manipulated p73 expression in HCC cells through overexpression and siRNA-mediated silencing.Changes in AXL expression were assessed via western blotting across different HCC cell lines.To evaluate AXL promoter activity, we used a luciferase reporter assay in TET-inducible p73-overexpressing HEP3B cells.Additionally, chromatin immunoprecipitation (ChIP) assays were performed to investigate p73 binding and histone modifications at the AXL promoter.

Result: Our investigation demonstrated a clear correlation between p73 and AXL expression. Overexpression and silencing of p73 led to corresponding changes in AXL levels. The luciferase reporter assay revealed that p73 overexpression significantly enhanced the transactivation of AXL, validating the functional role of the identified p73 binding motif in the AXL promoter. We detected enrichment of p73 to the AXL promoter through the ChIP assay. Additionally, the presence of specific histone modifications associated with transcriptional activation were detected via ChIP.

Conclusion: The findings prove the role of p73 as a key regulator of AXL expression in liver cancer cells. Since both p73 and AXL are significant in cancer progression, targeting the p73-AXL regulatory axis could provide new therapeutic strategies to overcome resistance in cancer treatment. This could lead to innovative approaches in managing tumors that are currently difficult to treat due to their resistance to conventional therapies.
This work was supported by TUBITAK grant No 123Z766

Keywords: HCC, AXL, p73

ST03

Autophagy is required for the development and functionality of lacrimal gland organoids

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Background/aim: Lacrimal glands (LGs) serve as pivotal exocrine glands crucial for protecting the surface of the eye through the secretion of water, electrolytes and defence proteins, including lactoferrin, lipocalin and lysozyme. A promising model to gain insight into the developmental and molecular mechanisms of LG formation and its secretory function is the generation of 3D LG-like organoids from human embryonic stem cells (hESCs). Autophagy is an evolutionarily conserved process that contributes to the development and maintenance of normal tissue function. Although basal autophagy is known to occur in the LG, it is still unclear how it affects LG development and its secretory function. We aimed to understand how autophagy affects LG development and secretory function.

Materials and methods: Here we have undertaken a genetic study by generating autophagy-deficient (ATG5-/-), hESC-derived LG-like organoids to investigate the impact of loss of autophagy on LG formation and secretion. We characterised the cellular and functional phenotypes of ATG5-/- hESC-derived LG-like organoids. We also evaluated the drugs for therapeutic interventions on ATG5-/- hESC-derived LG-like organoids.

Results: Our data indicate that autophagy is required for proper LG cell composition and secretion. Therapeutic interventions rescued the cellular phenotypes and improved functional phenotype.

Conclusion: This autophagy-deficient organoid model may provide a new angle for therapeutic interference in ocular diseases.

Keywords: autophagy, ATG5, organoids, lacrimal gland

ST04
SP1-mediated Up-regulation of MIR100HG in Hepatocellular Carcinoma

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Background/Aim: Long non-coding RNAs (lncRNAs) represent a category of RNA transcripts exceeding 200 nucleotides in length. Typically, lncRNAs lack the capacity to encode proteins or peptides. Over the past decade, it has become evident that a disrupted lncRNA profile is extensively implicated in the pathogenesis of various diseases, encompassing cancer, metabolic disorders, and cardiovascular diseases. Notably, lncRNAs have emerged as significant contributors to tumor growth and metastasis. MIR100HG is a non-coding RNA that is important in various cancer cells. There are gaps in the literature regarding how MIR100HG is regulated. Specifically, no studies have been found on its regulation by SP1. Therefore, we aimed to find out the regulation of MIR100HG by SP1 transcription factor.

Materials and Methods: Five different truncated promoter constructs, (-188/+231 (P1), -397/+231 (P2), -678/+231 (P3), -815/+231 (P4) and -1031/+231(P5), were generated by PCR based approach and cloned into luciferase reporter plasmids. Transient-Transfection studies were carried out using five different lengths of MIR100HG promoter constructs in Hep3B cells. Basal promoter activity was determined with luciferase reporter assay. Co-transfection assay was performed with SP1 expression vector. qRT-PCR analysis and western blotting analysis were carried out to determine the mRNA and protein expression studies, respectively.

Results: In a result of the study, the overexpression of SP1 was confirmed by mRNA and protein. The values obtained from SP1 overexpression were compared with basal promoter activities. It was determined that the SP1 transfection factor significantly increased luciferase activity in all constructs in a statistically meaningful manner. Especially, SP1 induced the luciferase activity of P2 promoter construct approximately four-fold. SP1 also upregulates MIR100HG mRNA expression.

Conclusion: These findings are novel in terms of Sp1-mediated the transcriptional regulation of MIR100HG, shedding light on fundamental molecular mechanisms that will be crucial in therapeutic strategies.

This project was supported by The Scientific and Technological Research Council of Turkey with the project number 121Z950.

Keywords: lncRNA, mir100hg

ST05

Unveiling the secrets of paclitaxel resistance and the key to overcoming this resistance in non-small cell lung cancer

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Background/Aim: Non-small cell lung cancer (NSCLC), the most common type of lung cancer, can be treated with surgery and chemotherapy. However, surgery alone is often insufficient to address metastasis, making chemotherapy the best treatment option. Initially, patients respond well to chemotherapy, but over time, chemoresistance can develop, often driven by tumor epigenetic alterations. To uncover the mechanisms behind chemoresistance, paclitaxel-resistant NSCLC cells were developed to identify novel ways to overcome this resistance and reveal the underlying processes.

Materials and Methods: Paclitaxel-resistant NSCLC cell lines were developed using a dose escalation method. Drug resistance was confirmed through SRB and colony formation assays comparing resistant and parental cells. Once resistance to paclitaxel was established, cell adhesion and doubling time properties were investigated. Additionally, the response of parental and resistant cells to platinum agents and other taxanes was evaluated using SRB assays. To uncover transcriptomic alterations between parental and resistant cells, RNA-seq was performed along with an epigenetic drug library screen to target potential epiregulators behind paclitaxel resistance.

Results: In summary, the relative resistance of paclitaxel between parental and resistant NSCLC cells was 40-fold, demonstrating successful development of resistant cells. Interestingly, there was only a slight difference in response to other platinum and taxane agents. The epigenetic drug library screen revealed that BRPF1 and HDAC inhibitors play key roles in targeting potential epiregulators in reversing paclitaxel resistance. RNA-seq indicated upregulated pathways in hypoxia and KRAS signaling in resistant cells, while Myc and E2F targets were downregulated. We are currently investigating the contribution of these pathways for the reversion of paclitaxel resistance and the key players in this process.

Conclusion: In conclusion, these findings, along with the identification of candidate epiregulators and transcriptomic alterations, could reveal novel agents that hold promise for reversing resistance in NSCLC, offering new avenues for effective treatments.

Keywords: Taxol, Chemoresistance, ABCB1, BRPF

ST06

Differential Salvage of Glutamine and Regulation of Lipid Metabolism in Primary versus Metastatic Colorectal Cancer Cells

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Background and Aim: Metabolic plasticity in cancer cells contributes to survival and metastatic advantages. L-glutamine, a non-essential amino acid, is crucial for cellular energetics in oxidative cells. This study aimed to evaluate the differential role of L-glutamine in metastatic versus primary colon cancer cells.

Materials and Methods: Differential expression of glutamine-related genes was determined in microarray data of 443 colorectal cancer (CRC) samples and 19 non-tumoral colorectal mucosas (GSE39582). Univariate Cox regression and hierarchical clustering were used to generate a gene signature for prognostication. Untargeted metabolomics and 18O based fluxomics were used to identify differential levels of metabolites and energy turnover in paired primary and metastatic CRC cell lines SW480 and SW620. Western blot and qRT-PCR were used to validate differential gene expression. Subcellular localization of E-cadherin was determined by immunocytochemistry. Lipid droplets were visualized with Nile red.

Results: The GO term "Glutamine metabolism" was significantly enriched in metastatic tumors versus primary tumors. A three-gene signature (FTCD, GGT1, LEP) associated with a worse prognosis was validated in multiple datasets. Both cell lines maintained high viability without L-glutamine, suggesting alternative energy sources. Untargeted metabolomics indicated the preferential accumulation of lipids in SW620 but not SW480 cells. Nile Red staining indicated more lipid droplets in SW620 cells upon L-glutamine starvation. Inhibition of fatty oxidation or synthesis did not reduce lipid droplet formation in SW620 cells, indicating reliance on fatty acid uptake. Additionally, L-glutamine starvation led to the formation of buds in spheroid structures in SW620 cells, suggesting enhanced metastatic behavior.

Conclusion: Metabolic reprogramming in cancer cells involves enhanced lipid biosynthesis or oxidation, supporting survival and metastasis through various mechanisms. L-glutamine scarcity activates compensatory fatty acid metabolism, providing metastatic advantages to cancer cells. Understanding these metabolic vulnerabilities could improve therapeutic outcomes.

Keywords: fatty acid metabolism, metastatic behavior, L-glutamine

ST07

Searching for the roots of vertebrate cortical evolution in zebrafish forebrain

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Adult of many teleost fish species, including zebrafish with well-developed pallium can perform cognitively demanding associative, spatial, social learning and working memory tasks. Hence, zebrafish must have evolved a version of pallial circuit elements and architectures that can enable cognitively demanding behaviors, by using the raw cellular constituents and connectivity principles that were available to the ancestors of all vertebrates. While homologs of mammalian amygdala, olfactory cortex, and hippocampus in zebrafish pallium have been proposed, how exactly the cellular elements of teleost pallium relate to vertebrate cortical evolution, has not been answered. We generated the first sub-cellular resolution Atlas of Zebrafish Transcriptomic Encephalic Cytoarchitecture (AZTEC) of 99 multiplexed genes for >300.000 neurons. Our results revealed multiple excitatory and inhibitory neuron types and non-neuronal cells across zebrafish forebrain and propose distinct marker genes. We observed that while some of these cell types are dispersed widely, several inhibitory and excitatory neuron classes are organized into spatially distinct forebrain nuclei. Spatio-molecular clustering of AZTEC data and 3D alignment onto adult zebrafish brain atlas confirm several previously proposed forebrain nuclei, but also reveal multiple novel substructures that were not described. Aligning AZTEC with single-cell transcriptome of zebrafish and other vertebrates revealed several cell types (non-neuronal, excitatory, inhibitory) and cortical/subcortical regions that are conserved across vertebrates. Evolutionarily identified zebrafish forebrain regions mapped by AZTEC exhibit spatio-temporally distinct resting-state activity and functional connectivity, measured by calcium imaging in adult and juvenile zebrafish.

Keywords: evolution, brain, zebrafish, spatial biology, bioinformatics

ST08

A complete gene regulatory network underlying neuromesodermal progenitor cell fates in zebrafish

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Background and Aim: The neural crest (NC) is a multipotent cell population contributing to craniofacial structures, peripheral nervous system (PNS), and body pigmentation in vertebrate embryos. NC cells delaminate from the dorsal neural tube, and their fate is dictated by their anterior-posterior axis location. Neuromesodermal progenitors (NMPs) are a distinct posterior embryonic cell population giving rise to paraxial mesoderm and central nervous system (CNS) lineages. In vitro studies using pluripotent stem cells show that trunk-like NC derivatives require an intermediate state expressing both NC and NMP markers. The in vivo link between trunk NC and NMPs remains elusive, raising questions about the role of NMPs in specifying trunk peripheral neuronal populations, traditionally thought to derive from NC.

Materials and Methods: We employed a single-cell genomics approach to analyse NMP fates during embryonic and larval development in both wild-type and foxd3-mutant conditions. We built developmental trajectories using dynamic velocity approaches and validated them with inducible Cre transgenic genetic lineage tracing, coupled with single-cell sequencing. We used the SCENIC+ framework to analyze our single-cell multiomics data to generate predictable gene regulatory networks (GRNs) underlying critical NMP cell fate transitions. We analysed the most relevant regulons using CRISPR knockouts and single-cell resolution Perturb-seq.

Results: We identify an unprecedented range of NMP derivatives. NMPs activate axial and neural programmes, differentiating into CNS neurons and glia, skeletal and muscle elements, myeloid, vasculature and many others. Notably, we find contributions to the PNS, including sympathetic, enteric, and autonomic neurons, previously thought to be entirely NC-derived. We identify and probe gene regulatory networks (GRNs) underlying different NMP derivatives, identifying specific drivers of NMP cell fate transitions.

Conclusion: Here, we decipher and test comprehensive predictive NMP GRN as a cell fate paradigm in vertebrate embryos. Our study highlights the potential application of newly discovered programs for NMP derivation to generate PNS derivatives, thereby advancing prospects for cell therapies.

Keywords: neuromesodermal progenitors, gene regulatory network, developmental biology, zebrafish, neural crest

ST09

High-Throughput analysis of the content and properties of nano-sized bioparticles with single-particle profiler

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Background: Flow cytometry is universally applied to screen the content and properties of single cells in high-throughput manner. However, for single biological particles in human blood (e.g. lipoproteins or extracellular vesicles) it is challenging due to their small (10 - 100 nm) sizes.

Materials and Methods: Here, we developed a method called "Single Particle Profiler" (SPP) which utilizes the "spot scanning" modality of confocal microscope to record fluorescence intensities of fluorescently labelled single particles that are freely diffusing in the solution through the observation volume. Two- and multicolour profiling is possible which allows for extracting information on content of biological particles. Moreover, we utilize the so-called "smart probes", sensitive to the membrane environment to profile biophysical properties of membranes in extracellular vesicles and lipid assemblies in lipoproteins.

Results: First, we tested the SPP performance using artificial liposomes (large unilamellar vesicles - LUVs, 100 nm in diameter). Then, we applied SPP to study various lipid particles in human body. We used SPP for quality control of RNA encapsulation in lipid nanoparticles (LNPs), we explored antibody binding to virus-like particles (VLPs) to study the immune response to virus infection and finally we explored the biophysics of lipoproteins and extracellular vesicles in health and diseases.

Conclusion: SPP is a promising method for high-throughput screening of biological nanoparticles in single-particle manner, it has potential in biomedical and biotechnological applications as it provides capabilities for quality control of products for drug delivery, single particle content screening as well as for discovering novel biophysical disease biomarkers.

Keywords: Liposomes, exosomes, viruses, lipid nanoparticles, single particle profiling

ST10

Establishment and 3D-Image-Based Profiling of Drug Responses in Patient-Derived Organoids from Metastatic Colorectal Cancer

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Backgroud/Aim: Several studies have shown that tumors can retain certain features in three-dimensional patient-derived organoids (PDOs). When considering the heterogeneity of colorectal cancer and the ability of PDOs to mimic their parental tumors, PDOs are powerful for drug profiling via high-throughput screening approaches. Moreover, this platform is useful for ex vivo evaluation of targeted agents and chemotherapeutics in personalized therapy. In this study, we aimed to establish Türkiye's first living PDO biobank from 20 heavily pretreated metastatic colorectal cancer patients (mCRC), encompassing morphological and genomic characteristics and drug sensitivities.

Materials and Methods: To assess phenotypic similarities of the PDOs with their corresponding parental tumors, hematoxylin-eosin staining was utilized. The genomic profile of the different patients, for both parental tumors and their matched PDOs, was performed using Whole-Exome Sequencing (WES) and RNA sequencing (RNA-Seq). Then we proceeded with drug screening to identify anti-cancer drug sensitives in a 3D image-based approach by evaluating organoid volume, nuclei numbers, and proliferation index through EdU incorporation assay.

Results: The histologic characteristics in the PDOs have been validated by the observations performed by the independent pathologist. WES and RNA-Seq analyses on PDOs and matched tumor revealed a high degree of consistency in mutation and copy number variation and maintenance of the transcriptomic signature between them. The drug screening using a library of 50 compounds was demonstrated to be functional testing of effective hits in PDOs, indicating the platform's potential use and expansion into clinical decision-making.

Conclusion: In conclusion, we established a living biobank of PDOs from 20 patients with mCRC, including genomic and drug sensitivity analyses. Moreover, we implemented a protocol for running image-based 3D-high-content screenings on PDOs and we successfully applied this pipeline to test 50 drugs. These findings indicate that our PDO biobank, combined with image-based screenings, are effective tool for translational research and eventually for developing personalized medicine.

Keywords: Metastatic colorectal cancer, High-Throughput Screening, Patien-derived Organoids

ST12

Investigation of the Role of Wnt/B-Catenin Signaling in Development of Alzheimer's Disease in a Zebrafish Model of Amyloid-β Toxicity

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Background/AIM: The Wnt/β-catenin signaling pathway, an evolutionarily conserved and crucial pathway associated with synapse formation in adulthood, plays a pivotal role in Alzheimer's disease (AD). AD, marked by various pathologies, is primarily linked to the accumulation of extracellular beta-amyloid plaques. The interplay between this accumulation and disruptions in the Wnt/β-catenin signaling pathway triggers synaptic degeneration, resulting in synaptic dysfunction and AD progression.

Materials and Methods: In this study, we modeled AD induced by the Aβ42 peptide using adult transgenic (6XTCF) zebrafish. To establish the zebrafish AD model, we employed cerebroventricular microinjection (CVMI) with the Aβ42 peptide. Fish, anesthetized prior to CVMI, were positioned on a stable platform, and the Aβ42 peptide was injected into the telencephalon region of the brain by a capillary needle. Brain samples were collected on 1, 3, 4, 7, and 14 days post-CVMI (dpi) to analyze changes in Aβ42 peptide accumulation, the immune system response, synaptic degeneration, apoptosis, and the expression of genes related to proliferation using qPCR and immunofluorescent staining. To examine the role of the Wnt/β-catenin signaling pathway in the molecular mechanism of AD development, fish exhibiting high levels of regeneration on days 7 and 14 were treated with the IWR1 drug, which inhibits Wnt/β-catenin signaling by stabilizing the Axin2 protein, thereby suppressing the regenerative response.

Results: Our results revealed that the AD model manifested on 3 dpi, with the regenerative response reaching its peak on 7 dpi and 14 dpi. Treatment with IWR1 resulted in increased Aβ42 accumulation, accelerated synaptic degeneration, and elevated cell deaths in fish where the Wnt signaling pathway was inhibited.

Conclusion: Our adult zebrafish AD model is poised to elucidate the molecular mechanisms connecting the Wnt signaling pathway and AD, thereby contributing to the development of alternative therapeutic approaches for AD patients.

Keywords: Alzheimers Disease, wnt-b catenin pathway, zebrafish, cvmi, Beta-amyloid plaques

ST13

Identification of Neuronal Membrane Proteasome-Derived Peptides That Modulate Neuronal Signaling

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Background/Aim: Protein homeostasis is essential for maintaining cellular health. Proteins are synthesized and eventually degraded to balance protein levels in all cells. The main non-lysosomal protein degradation machinery in eukaryotic cells is the proteasome, a large macromolecule equipped with proteases responsible for the breakdown of proteins and production of peptide fragments. Strikingly, exclusively in neurons, the proteasome can localize to the plasma membrane, where it degrades newly synthesized intracellular proteins into extracellular peptides. This unique co-translational degradation mechanism highlights a distinct role for neuronal membrane proteasomes (NMPs). Selective inhibition of the NMP complex halts peptide release and rapidly attenuates neuronal transmission. Based on these findings, we hypothesized that NMP peptides mediate a novel form of communication through specific peptide-receptor interactions to promote intracellular signaling cascades relevant to neuronal development and function.

Materials and Methods: We investigate the function of the NMP complex and its resulting peptides using various biochemical assays, including activity-based probes, surface biotinylation, peptide isolation and purification, as well as imaging and omics techniques.

Results: Our work indicates that purified NMP peptides, once applied to naïve neurons, rapidly induce N-methyl-D-aspartate receptor (NMDAR)-dependent calcium influx, leading to sustained phosphorylation of the activity-dependent transcription factor cAMP response element-binding protein (CREB). We also determined that the gene expression program and the proteome pool drastically change upon NMP peptide treatment with increased expression of activity-regulated genes (e.g., Fos, Npas4, Egr4) known to have critical neuroregulatory roles.

Conclusion: This novel pathway is parallel to classic neuronal activity-dependent programs and points to NMP and its resulting endogenous peptides as potential key modulators of neuronal function in health and disease. Further elucidation of the regulatory mechanisms governing the NMP complex and identification of its bioactive peptide products are critical steps toward fully understanding this novel signaling process in the mammalian nervous system.

Keywords: proteasome, activity-based probes, protein degradation, neuronal signaling, neurodegeneration

ST14

Investigation Into The Role Of Patl1 Gene In Neurodevelopment Using Drosophila Melanogaster As Model Organism

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Background/Aim: Neurodevelopmental disorders (NDDs) are characterized by limitations in adaptation, social behaviour, and intellectual disability (ID). The fruit fly Drosophila melanogaster serves as an ideal model to study the genetic causes of NDDs allowing for screening of candidate genes with straightforward analyses. We report a variant in the PATL1 gene in a patient diagnosed with walking difficulties and ID, suggesting that the PATL1 gene is a candidate gene for NDDs. PATL1 is a P-body protein that is critical for the spatiotemporal regulation of mRNAs in various cell types, and this regulation is particularly essential in neurons for plasticity and response to stimuli. Previously, the PATL1 ortholog in fruit flies, Hpat, was shown to cause synaptic defects when mutated and is crucial for synaptic plasticity. In this study, we aim to determine the role of PATL1 in neurodevelopment, motor functioning and learning and memory by using the fruit fly model.

Materials and Methods: Loss-of-function analyses were carried out using Hpat null and hypomorphic mutants, along with tissue-specific RNAi experiments. To model the human disease-causing PATL1 variant, we generated flies expressing wild type and mutant PATL1. Morphological analyses were conducted via immunohistochemistry, focusing on the learning and memory center, the Mushroom Body (MB). Behavioral outcomes were assessed at the larval stages through the larval crawling assay.

Results: Our results indicate that Hpat ablation is lethal, while neuronal knockdown causes semi-lethality accompanied by severe motor dysfunction. We demonstrate that Hpat is necessary for the development of the MB and plays critical role in the laminar organization of the ellipsoid body, the fly's counterpart to the human basal ganglia. Behavioural analyses revealed early-stage motor defects impairing larval locomotion, exploratory behaviour, and synaptic growth. This results mimics the pathology of PATL1 variant. Morphological, molecular, and behavioral data from “humanized” transgenic flies are expected to provide insights into the etiology of PATL1 linked disorders.

Keywords: Neurodevelopmental disease, animal models, Drosophila melanogaster, P bodies, PATL1

ABSTRACTS
POSTER SESSION

P001

A Weight-Induced Sparse Regression Algoritm to Deconvolute Cell-type Medleys in Spatial Transcriptomics Data

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Background/Aim: The state-of-the-art tools of modern science have turned to collecting large and complex data sets with the increase in technological developments and innovations. By using single cell RNA sequencing (scRNA-Seq) methods as today's cutting-edge technology in molecular biology, cell heterogeneity of cell populations can be obtained with high resolution through gene expression data at the cellular level. However, this technology cannot detect the spatial positions of cells. On the other hand, spatially-resolved gene expression profiles obtained from spatial transcriptomics (ST) are playing key role to understand tissue organization and function. But ST technology lacks single cell resolution. Here, the aim is to deconvolute spatial cellular profiles from ST data.

Materials and Methods: We developed a machine learning model, namely weight-induced sparse regression (WISpR), that leverages high resolution cell-type data from scRNA-Seq to deconvolute profiles from ST, which serves spatial locations of cellular information in a lower resolution. WISpR employs a spot-specific hyperparameter optimization technique to enhance the robustness of its predictions together with sequential thresholding for maintaining the true biological sparsity of the system.

Results: The performance superiority of WISpR was benchmarked against 5 recently published models using 4 matched and 6 blended simulated datasets. Moreover, WISpR was applied on 2 healthy datasets as the developing embryonic human heart and mouse brain and correctly mapped the cell profiles and defined overall tissue architecture, successfully. Finally, WISpR revealed the zone-specific cellular cancer heterogeneity in human breast cancer.

Conclusion: Overall, the principle of WISpR was driven by the sparsity of nature and it will provide promising contributions to personalized medicine as it enables the high-resolution molecular profiling of biological tissues.

Keywords: Deconvolution, Machine Learning, Transcriptomics

P002
In Vitro Anti-Cancer Activity of Novel Curcumin Loaded PLGA-DSPE Hybrid Nano Drug Delivery System

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Background/Aim: Poly(D,L,lactic-co-glycolic acid) (PLGA) stands as an FDA-approved biopolymer recognized for its biocompatibility, biodegradability, and manufacturing convenience, rendering it an ideal candidate for transporting hydrophobic drugs. Despite its promising therapeutic effects in cancer treatment, curcumin, derived from turmeric rhizomes, faces challenges due to its low water solubility and in vivo biodistribution complexities. This study seeks to augment the hydrophobic capacity of PLGA for curcumin delivery by integrating DSPE, an FDA-approved phospholipid lauded for its biocompatibility. Despite significant strides in cancer treatment with chemotherapeutics, drug resistance remains a major obstacle in the clinical application of agents such as 5-fluorouracil (5-FU), particularly in colon cancer therapy. Combination therapy strategies offer a promising approach to overcome this limitation, potentially enhancing safety and efficacy compared to monotherapy regimens, especially when combining drugs with distinct modes of action.

Materials and Methods: In this study, a nano drug delivery system was developed employing a combination of double emulsion solvent evaporation (w/o/w) and film-forming techniques, with curcumin loaded into the hybrid carrier at 250 µg/ml concentration. Toxicity and activity evaluations of the synthesized nanoformulations were carried out using colon adenocarcinoma (LoVo) and healthy fibroblast (CCD-1072Sk) cell lines. The
in vitro cell culture assays investigated the cytotoxic, apoptotic, ROS-generating, and synergistic effects of the nanoformulation in combination with 5-FU.

Results: The findings of the study reveal the elevated cytotoxic potency of the combined treatment relative to individual interventions. Moreover, statistical analysis reveals a significant increase in the effect of the optimized curcumin-loaded PLGA-DSPE hybrid formulation on apoptosis and ROS production compared to free curcumin.

Conclusion: Compared to free curcumin, it was ensured that hybrid nanoparticles provided better dispersibility in aqueous media, increased stability, and enhanced the curcumin-carrying capacity of PLGA. Thus, it has been shown that the effectiveness of chemotherapy is increased in nano-curcumin and 5-FU combination therapy, with increased cellular toxicity, apoptosis, and ROS level.

Keywords: curcumin, hybrid nanoparticle, PLGA, Cancer, Drug delivery

P003
Lysosomal signalling lipid Phosphatidylinositol 3,5-bisphosphate safeguards yeast longevity

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Background/aim: PI(3,5)P2 (phosphatidylinositol 3,5-bisphosphate) is a conserved signaling lipid of the endo/lysosomal system. PI3,5P2 is synthesized through phosphorylation of PI3P by the lipid kinase Fab1 (PIKfyve in mammalian cells). Vac14 (ArPIKfyve in mammalian cells) and Vac7 are essential proteins required for Fab1 activity, whereas PI(3,5)P2 binding protein Atg18 is a negative regulator of Fab1. PI3,5P2 is mainly synthesized on the membrane of the yeast vacuole and is involved in various cellular processes. Data from our lab suggests cell cycle-related functions for PI(3,5)P2 in yeast, yet whether PI(3,5)P2 is present in cycling cells under basal conditions remains elusive. Here, we investigate how PI(3,5)P2 is distributed during the cell cycle and how it contributes to yeast longevity.

Materials and methods: We generated yeast codon-optimized SnxA as an in-vivo sensor of PI(3,5)P2. Through fluorescence microscopy, we investigated the SnxA localization during the cell cycle. We also utilized a vacuole-directed in-vivo ratiometric pH sensor to measure vacuolar pH during the cell cycle. We created mutants with disrupted PI3,5P2 distribution and analyzed their vacuolar pH and chronological Life Span (CLS) in comparison to wild-type cells.

Results: During mitosis, PI3,5P2 accumulated on the vacuole that segregated to the daughter cell, while it diminished from the vacuole that stayed in the mother cell. This coincided with an increase in the daughter's vacuole acidity and a decrease in the mother's. A hyperactive mutant of Fab1 (fab1-ha) resulted in the symmetrical distribution of PI3,5P2 on both vacuoles and caused acidification of the mother vacuole. In contrast, the deletion of ATG18 also resulted in a more symmetrical distribution of PI35P2 but caused a significant decrease in vacuole acidity, which could not be rescued by (fab1-ha). CLS results showed that cells harboring fab1-ha had a longer life span.

Conclusion: Our results establish PI3,5P2 asymmetry as a key player in asymmetric cell division of budding yeast and yeast life span.

Keywords: PI35P2, Chronological Life Span CLS, Cell Cycle, Vacuolar pH

P004
Exploring the In Vitro Applicability of Raman Spectroscopy for Drug Screening on Non-Small Cell Lung Cancer

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Background/Aim: Raman spectroscopy, renowned for its non-invasive and reliable nature, is increasingly investigated for its potential in *in vitro* cellular studies and clinical research worldwide. This study aimed to explore the utility of Raman spectroscopy as a label-free method for cell screening, specifically focusing on elucidating molecular changes in non-small cell lung cancer (A549) and normal lung epithelial (BEAS-2B) cell lines upon drug exposure.

Materials and Methods: Two chemotherapy drugs, Olaparib and Gemcitabine, were administered at increasing concentrations, and their therapeutic index (TI) values were determined using the resazurin cell proliferation assay. Molecular changes were monitored at 24, 48, and 72-hour time points following drug exposure using Raman spectroscopy with a 785 nm wavelength laser.

Results: At 72-hour incubation, IC_{50} values were found for Olaparib (110.92±5.8 µM for A549, 179.32±6.3 µM for BEAS-2B) and Gemcitabine (161.09±8.2 nM for A549, 338.72±6.6 nM for BEAS-2B), yielding TI values of 2.1 and 1.62, respectively. Molecular screening at 24-hour incubation using concentrations near the IC_{20} values of both drugs in the A549 cell line (50 µM for Olaparib, 25 nM for Gemcitabine) revealed changes in lipid and protein levels compared to the BEAS-2B cell line.

Conclusion: Raman spectroscopy demonstrates its potential for determining concentration-dependent molecular changes upon drug exposure even at earlier time points. This biochemical insight into drug mode of action offers researchers a valuable tool for high-sensitivity and high-specificity drug screening and comparison.

Keywords: Spectroscopy, Label-free analysis, Single-cell analysis

P005
The effect of inflammatory cytokines: IL-6 and TNF-α on SMN1 gene expression

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Background/Aim: SMN1 (Survival Motor Neuron-1 gene) synthesizes a 38 kD protein and is associated with Spinal Muscular Atrophy (SMA), a disease resulting from the death of motor neurons. It has been identified in BV-2 (microglial cells) as a gene that negatively regulates the NF-κB signaling process. In the literature, there are no studies about the effect of IL-6 and TNF-α cytokines on the SMN1 gene in human neuroblastoma cells (SH-SY5Y). In this study, the impact of the inflammatory cytokines IL-6 and TNF-α on the SMN1 gene was analyzed using the qRT-PCR strategy.

Materials and Methods: For this purpose, SH-SY5Y cells were cultured in DMEM containing 10 % FCS. After culturing SH-SY5Y cells, cytokine treatment experiments were conducted at 24h and 48h time points. RNA isolation and cDNA synthesis were performed from the treated cells. Real-Time PCR analysis was carried out using specific primers for the SMN1 gene with the cDNA as a template. The data were analyzed using the Livak method.

Results: The results showed that the expression of the SMN1 gene increased in SH-SY5Y cells treated with IL-6, while a decrease was observed in cells treated with TNF-α.
Conclusion: It is an important study that forms the basis for finding various target therapeutic molecules that regulate the SMN1 gene.

Keywords: SMN1, SH-SY5Y, IL-6, TNF- α

P006
In silico Investigation of the Atavistic Model of Cancer

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Background/Aim: Cancer manifests as a disruption in the regular functioning of multicellular systems, arising from the malfunctioning of genes responsible for cellular cooperation. Notably, cancer exhibits atavistic characteristics, wherein cancer cells diverge from the conventional Darwinian evolution, highlighting a distinctive trajectory in their evolutionary fate. Atavism refers to the re-emergence of ancestral traits or characteristics that have been dormant or suppressed in a species' evolutionary history. Cancer cells may display traits such as uncontrolled proliferation, evasion of cell death mechanisms, and altered metabolism, which could reflect ancient biological programs related to growth and survival. Herein, we aimed to trace the evolutionary origin of cancer by analyzing tumor-related proteins in the basal metazoan Hydra and their preservation across diverse taxa.

Methods: To this end, we have employed bioinformatic approaches to investigate the conservation status of protein-coding genes that are differentially expressed in the tumor-bearing Hydra across species, representing both unicellular and multicellular forms of life.

Results: By examining the taxonomic distribution of the Hydra polyp-related proteins, we have traced the ancient evolutionary roots of cancer through the tree of life. The majority of protein-coding genes were found to be of unicellular origin and associated with cancer.

Conclusion: We suggest conservation of the atavistic model of cancer in the basal tumor-bearing animals. We also propose Hydra as a promising model organism in cancer research, shedding light on the evolutionary underpinnings of cancer and highlighting its relevance in broader biological contexts.

Keywords: Atavism, Cancer, Evolution, Phylogeny, Bioinformatics

P007
Development of a Green Fluorescent Protein (GFP)-based Cellular Reporter to Measure the Effects of Food Additives on Obesity-Related Genes

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Obesity is a significant risk factor for numerous diseases. The prevalence of obesity has started to increase with changes in our lifestyles. Processed foods containing food addives are considered contributing factors. Studies have shown that these dietary components can influence gene expression through epigenetic mechanisms, such as DNA methylation in promoter and other gene regulatory regions, potentially affecting disease susceptibility.

In this study, we aimed to test possible epigenetic effects of a sucralose-based sweetener on two obesity-associated genomic loci, which were identified in obesity genome-wide association studies (GWASs) and localized near the genes, MSI2 and CPT1A. For this purpose, we designed reporter vector plasmids containing these regulatory regions near the MSI2 and CPT1A genes and cloned upwnstream of a GFP reporter protein. The vectors were transfected into human embryonic kidney cells (HEK293T) and cultured with different concentrations of the sucralose-based sweetener. We observed an increase in GFP signal compared to control samples, which indicate possible epigenetic effects of the sweetener on these two loci associated with obesity.

Our findings suggest that sucralose-based sweeteners may induce methylation and/or other epigenetic changes in the regulatory regions of these obesity-related genes, leading to altering of their expression. This study also provide proof-of-principle for the design of similar reporters to assess the effects of environmental factors on disease-associated gene regulatory loci. Further epigenetic analyses, such as bisulfite sequencing and methyl-specific qPCR, are warranted to elucidate these effects comprehensively.

Keywords: MSI2, CPT1A, Obesity, Sweetener, Reporter Assay

P008
A Human Cell-Based 3D In Vitro Glioblastoma Microfluidic Model Integrated With Blood-Brain-Barrier Functionality

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Background/Aim: Glioblastoma (GBM) is a highly malignant and aggressive brain tumor originating from glial cells, with rising incidence worldwide. Despite various therapeutic approaches, the main challenge in GBM treatment is the blood-brain barrier (BBB), which protects the brain in health but limits drug delivery and alters in disease. To understand the BBB state in GBM, there is a pressing need to create more physiologically relevant human cell-based 3D brain disease models.

Materials and Methods: We developed a GBM-BBB-on-a-chip model consisting of two compartments: the brain compartment with U87 glioblastoma cells, primary human astrocytes and pericytes, and the blood compartment with induced pluripotent stem cell (iPSC)-derived brain-like microvascular endothelial cells (iBMECs). The chip models were maintained under dynamic media flow or static conditions in the blood channels, while the brain channels were always kept under static conditions. To assess the impact of GBM on the BBB, the dynamic or static models were developed for various glioblastoma cell densities and characterized for essential biomarkers via immunostaining, barrier integrity through trans-endothelial electrical resistance (TEER) measurements, and dextran permeability assays. Additionally, we performed experiments with astrocyte-conditioned media on U87 cells to observe their proliferation differences in the presence of secretions of glial cells.

Results: The dynamic GBM-BBB-on-a-chip models showed decreased BBB integrity, closely resembling
in vivo GBM models, whereas static GBM-BBB-on-a-chip models demonstrated increased BBB integrity. The study also highlighted behavioral changes in GBM proliferation and emphasized the importance of co-culturing GBMs to mimic the disease model.

Conclusion: Our model offers a promising platform for studying novel drug delivery approaches for GBM treatment, investigating the evolution of the GBM-tumor-microenvironment, understanding the mechanisms of intercellular communication within this environment, and exploring the potential dissemination of GBM cells throughout the secretion of various factors, which may serve as potential biomarkers for early GBM detection.

Keywords: iBMECs, Glioblastoma, Blood-Brain-Barrier, GBM-BBB-on-a-chip

P009
Evaluation of Mutations in Autosomal Dominant Polycystic Kidney Disease using Next Generation Sequencing Technology

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Background/Aim: Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary kidney disease. It is characterised by the progressive formation of kidney cysts, often with symptoms as kidney pain, bleeding and hypertension. In most cases, mutations in *PKD1* and *PKD2* genes are responsible for ADPKD. *PKD1* truncating mutations typically correlate with rapid disease progression, whereas mutations in *PKD2* gene lead to a slower progression. Furthermore, in recent years, mutations in over 30 genes have been associated with ADPKD. The pathophysiological mechanism of ADPKD has not yet been fully understood. Our aim is to investigate novel variations in the *PKD1* and *PKD2* genes, as well as explore new candidate genes that may contribute to the development or progression of ADPKD.

Materials and Methods: Whole exome sequencing (WES) analysis was performed using next-generation sequencing technology on peripheral blood samples from the 50 ADPKD patients. The WES procedure was performed and ADPKD-related gene variations were evaluated using the SOPHIA DDM platform. Detected variants were assessed and classified based on their pathogenicity as pathogenic (P), likely pathogenic (LP), or variants of uncertain significance (VUS), following the criteria outlined by the American College of Medical Genetics and Genomics.

Results: We identified a total of 14 P, 12 LP and 36 VUS mutations in *PKD1* and *PKD2* genes, with 20 of these mutations being novel. Additionally, there were a total of 37 gene variations detected in other ADPKD-related genes. Beside novel mutations in *PKD1*, mutations in the *CYP21A2*, *CLDN16*, and *CFTR* genes, identified as new candidate genes, were validated using Sanger sequencing. Currently, we are investigating the correlation between these identified gene mutations and the progression of ADPKD in patients.

Conclusion: Identifying novel gene variations in the *PKD1* and *PKD2* genes and evaluating ADPKD-related gene mutations are crucial for understanding the pathophysiology of ADPKD.

Keywords: Autosomal dominant polycystic kidney disease, PKD1, PKD2, whole exome sequencing

P010
Investigation of Anticancer Mechanisms of Thioridazine and Etoposide Combination in Papillary Thyroid Cancer

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Aim: This study aimed to investigate the anticancer activity and molecular mechanisms of thioridazine hydrochloride, an antipsychotic drug, in combination with etoposide in TPC-1 human papillary thyroid cancer cells. The goal is to address the resistance observed in thyroid cancer to etoposide, emphasizing the importance of identifying new treatment targets and developing innovative treatment approaches. Exploring alternative uses of FDA-approved non-cancer drugs, such as repurposing antipsychotic medications for cancer therapy, offers potential advantages in overcoming the challenges associated with time-consuming and expensive drug development processes.

Materials and Methods: SRB viability analysis was conducted to evaluate the cytotoxic effects of etoposide and thioridazine hydrochloride. The mode of cell death in cancer cells was determined using triple fluorescent staining and z-VAD-FMK inhibitor application. Colony formation analysis was performed to investigate the colony-forming ability. The actin cytoskeleton organization of the cells was observed using the Phalloidin-FITC fluorescence staining technique.

Results: Our results showed that the combination of thioridazine hydrochloride and etoposide effectively inhibited cell growth in TPC-1 cells compared to individual drug treatments. The triple fluorescent staining results indicated that the combination treatment induced apoptosis in the cells. Additionally, the preservation of cell viability with z-VAD-FMK application suggested that the combination therapy induces apoptosis, leading to cell death. The colony-forming ability of TPC-1 cells was reduced upon combination treatment, as evidenced by the results of the colony formation analysis. Moreover, phalloidin-FITC fluorescence staining revealed a significant decrease in cell density and disorganization of actin filaments in the cytoskeleton of the cells following the combination treatment, indicating induction of cell death by cell cytoskeleton disruption.

Conclusion: The thioridazine-etoposide combination therapy has demonstrated significant anticancer potential in thyroid cancer cells.
This study was supported by the Scientific Research Projects Unit of Bursa Uludagğ University (project number: FHIZ-2024-1834).

Keywords: Thioridazine-HCL, Etoposide, Thyroid cancer, Drug repurposing

P011
Investigation of the Effect of Cu(II) Flavonoid Complex on Apoptosis and Epithelial-Mesenchymal Transition (EMT) in Colorectal Cancer

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Background/Aim: Colorectal cancer, a prevalent and potentially fatal form of cancer, originates in the colon/rectum. Despite the use of many chemotherapeutic agents in the clinic, the observation of metastases resulting in recurrence in 50% of the patients after treatment makes the treatment process very difficult. New studies are being conducted with natural substances such as flavonoids. This study aims to investigate the potential effect of the copper (Cu) (II) flavonoid complex ([Cu(queH-1) Cl(phen)].2.5H_2O] queH=quercetin, phen=1,10-phenanthroline) on the mechanism of cell death and the EMT process.

Materials/ Methods: The effect of Cu(II) flavonoid complex on HCT-116 and HT-29 colorectal cancer cells was tested using SRB viability analysis. The cell death mode was investigated via triple fluorescence staining (Hoechst 33342, Annexin-V, and Propidium Iodide), M30 Elisa, and flow cytometry analyses. The apoptotic cell death was further investigated using Z-VAD-FMK inhibitor treatment. Anti-metastatic characteristics of the complex were studied through wound healing and invasion assays. The expressions of apoptotic and EMT markers were measured using western blot analysis.

Results: The Cu(II) flavonoid complex demonstrated a significant ability to induce cell death through apoptosis in colorectal cancer cells. However, it did not cause any significant decrease in cell viability of healthy colorectal cells (CCD-18-Co) at the same doses, suggesting it is specific for cancer cells. The apoptotic nature of the cancer cells after complex treatment was confirmed through pan-caspase inhibitor studies. Furthermore, wound healing and invasion assays validated its potential as a metastasis inhibitor. The examination of signaling pathway proteins yielded substantial evidence supporting the role of the Cu(II) flavonoid complex in stimulating apoptosis and inhibiting EMT.

Conclusion: These findings suggest that the Cu(II) flavonoid complex holds significant promise as a potent chemotherapeutic agent with anti-apoptotic and anti-metastatic properties in colorectal cancer. Further investigations are warranted to explore its therapeutic potential comprehensively.

Keywords: Colorectal cancer, EMT, Flavanoids, Quercetin, Phenanthroline

P012
Study Of the Circadian Clock Gene Bmal1 and the PPARã Gene Responsible for Adipogenic Differentiation In UV-Applied Stem Cells

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Absract
Mesenchymal stem cells (MSC) are multipotent cells that produce cytokines with the potential for cellular differentiation and are used in self-renewal and tissue healing. BMMSCs are derived from bone marrow, show low immunogenicity, and multidirectional differentiation. MSCs of adipogenic origin are derived from adipose tissue and are unaffected by donor age. Circadian rhythm is the daily regulatory system in eukaryotic organisms and cells. Bmal1 protein is involved in the circadian rhythm cycle. Knockdown of Bmal1 changes differentiation potential in stem cells by abnormal induction of the differentiation gene *in vitro* and is required for cell differentiation. PPAR-γ represses Bmal1 transcription and expression in adipocytes by triggering downregulation of glutamine and methionine uptake. Therefore, PPAR-γ integrates the adipocyte clock and regulates circadian rhythm through epigenetic regulation of Bmal1. Ultraviolet (UV) and visible light affect circadian rhythm regulation. UV causes DNA damage. When UV-induced DNA damage is not repaired, it can lead to mutations in the DNA sequence, especially those that disrupt the circadian rhythm. Various studies have shown that MSCs exposed to UV secrete more protective and healing secretions and as a result, their potential increases. In this study, we examined the effect of UV dose on cell differentiation and circadian rhythm in MSCs. The viability of MSCs exposed to UV light was analyzed by MTT. MSCs were cultured in adipogenic differentiation medium and Oil-red-o staining was performed on different days. On the 28th day, differentiation was most intense. MSCs UV-applied were then placed in adipogenic differentiation medium or not, for 28 days and the effect of UV on adipogenic differentiation and circadian rhythm was examined by RT-PCR. Statistical results showed that UV positively affected cell differentiation and circadian rhythm in ADMSCs and BMMSCs. It was also shown that adipogenic differentiation regulates circadian rhythm.

Keywords: Bmal1, Mesenchymal stem cell, PPAR, Ultraviolet light, RT-PCR

P013
Investigation Of The Cytotoxic Effect Of α-Tocopheryl Succinate (α-TOS) And SAHA Combination Treatment On SH-SY5Y Neuroblastoma cells

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Background/Purpose: Neuroblastoma is a malignant tumor developing from stem cells in the neural crest. Succinate dehydrogenase(SDH) is an essential mitochondrial enzyme in the synthesis of energy. Malfunctions of SDHA and SDHB genes hinder the functionality of SDH, leading to altered metabolism from mitochondrial energy production to glycolysis. This shift promotes the growth and spread of cancerous cells. This study seeks to clarify the molecular mechanisms of SDH inhibition by α-TOS, as well as the inhibition of the epigenetic regulator Histone deacetylase by Suberoylanilide Hydroxamic Acid(SAHA) in SH-SY5Y human neuroblastoma cells.

Materials and Methods: MTT(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell viability assay was used to evaluate the cytotoxic effects on SH-SY5Y cells in which α-TOS and SAHA were administered alone or in combination. Isobologram analysis was employed to determine synergistic effects and combination index(CI) values were calculated. Annexin V-FITC/PI staining was used to evaluate apoptosis. The long-term repopulation ability was assessed using colony formation assay.

Results: Isobologram analysis confirmed the synergism between α-TOS and SAHA, as demonstrated by CI values indicating enhanced cytotoxicity. α-TOS independently induces a dose-dependent necrosis, whereas SAHA primarily triggers apoptosis. 2μM and 4μM α-TOS induced necrosis in 5% and 30% of the cells, respectively. However, 25 μM of SAHA increased apoptosis by 10%. When combined, 4μM α-TOS and 25μM SAHA resulted in a 4-fold increase in necrosis and 3-fold reduction in apoptosis compared to control, highlighting their distinct and synergistic effects on cell death. Additionally, α-TOS+SAHA combination significantly reduced both the quantity and size of cell colonies, demonstrating the enhanced efficacy in inhibiting cellular proliferation.

Conclusion: This study demonstrates that α-TOS+SAHA combination significantly enhances cytotoxic effects in SH-SY5Y neuroblastoma cells synergistically, predominantly inducing necrosis and apoptosis and reducing long-term proliferative capacity. These findings suggest potential therapeutic strategies for targeting neuroblastoma using combined inhibition of SDH and HDAC enzymes.
This study is funded by TÜSEB (project no 28414).

Keywords: Neuroblastoma, Succinate Dehydrogenase, Mitochondria, Histone Deacetylases, Alpha-TOS

P014
The Novel Therapeutic Chalcone Complex Exhibits Anticancer Effects on Human Prostate Cancer And Prostate Cancer Stem Cells

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Background/Aim: Cancer stem cells (CSC), which is the main cause of the absence of the expected response in the treatment of prostate cancer and the development of resistance, are defined as self -renewing and transforming into many different cell types. Therefore, there is a need for studies proving that CSCs increase the risk of recurrence and metastasis in prostate cancer, and for new treatment approaches and drugs for prostate cancer. Due to their anti-cancer properties, chalcones and their derivatives show many anti-cancer effects such as suppressing tumor formation and inducing apoptosis. In this study, the cytotoxic and apoptotic effects of 4-hydroxy-3-methoxyphenyl chalcone complex, a chalcone derivative, were investigated on the human prostate cancer metastatic cell line (PC-3) and cell population rich in cancer stem cell population (cancer cells with CD133+/CD44+/CD24- antigen; prostatosphere) obtained from prostate cancer cells.

Materials and Methods: Surface markers of the created prostatospheres were characterized using the flow cytometry. The growth inhibitory effects of 4-hydroxy-3-methoxyphenyl chalcone complex were determined using the ATP assay. Combination studies with inhibitors of apoptosis, necrosis, necroptosis, oxidative stress and autophagy death pathways to determine the mode of cell death; triple fluorescent staining (Propidium iodide, Annexin-V and Hoechst 33342); Caspase 3/7 activity analysis was performed. Additionally, apoptosis at protein and gene levels was investigated by Western Blot and RT-PCR techniques.

Results: 4-hydroxy-3-methoxyphenyl chalcone complex has been proven to reduce cell viability and induce oxidative stress-induced apoptosis in both PC-3 cells and prostatosphere cells in a dose-dependent manner.

Conclusion: 4-hydroxy-3-methoxyphenyl chalcone complex was found to be cytotoxic in both PC-3 cells and prostate cells and induce oxidative stress-induced apoptosis. This in vitro study, in which we obtained promising findings, has the potential to guide us to understand whether it can offer new opportunities for therapeutic targets in further in vivo assays.
This study was funded by project number TGA-2023-1206.

Keywords: Prostate cancer, Oxidative Stress, Apoptosis, Prostatosphere, Chalcone

P015
Nanoparticle-based Vaccine Development Against Rotavirus with Multiple Pattern Recognition Receptor Ligands as Adjuvants

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Background/Aim: Rotavirus is a non-enveloped double-stranded (dsRNA) virus spreading through fecal-oral pathways and common cause of diarrhea-related death in children under 5 years of age worldwide. Even though immunity against virus through vaccine can be developed in early lifetime, the rate of vaccination is still low due to concerns such as safety of live attenuated vaccine, gut related side effects, and insufficient protection in developing countries. Therefore, we aim to develop of a new safer local antigen-containing subunit vaccine with multiple pattern recognition receptor agonists as adjuvants. However, neonate innate immunity is different than adult. Therefore, we require adjuvants which will respond in early life too. Our goal is to design better vaccines by understanding the expression and activation of PRRs within different development stages of life.

Materials and Methods: To test effectiveness and interaction between multiple PRR agonists in neonates, splenocytes of BALB/c isolated from neonates (6-7 days) or adults (6-8 weeks) were stimulated with single and multiple PRR agonists. Secreted cytokine amounts (IL-6, IL-12, IFN-γ, and IFN-a/β) were determined using ELISA and B16 Blue reporter cells using supernatant of treated cells. Upregulation of co-stimulatory (CD80 and 86) on cells was checked by flow cytometry after staining with appropriate fluorochrome-conjugated-antibodies. Finally, antigen processing capacities of splenocytes were detected via stimulating with adjuvants together with DQ-OVA. Then, processed model antigen OVA was determined via flow cytometry.

Results: Our results demonstrated that combining TLR9 and STING or TLR3 agonists raised IFN-γ and IFN-a/β levels in neonate mice though they were not responding to single agonists. Furthermore, co-stimulatory molecules were elevated, and co-inhibitory molecules decreased. Lastly, DQ-OVA processing increased in combination group compared to untreated splenocytes, which indicates effectiveness of adjuvants for vaccine development.

Conclusion: Next, we are planning to vaccinate both neonates and mothers to compare effectiveness of our vaccine at difference age groups.

Keywords: Adjuvant, Innate Immunity, Pattern Recognition Receptors, Vaccine

P016
The Inhibitive Effect of Atiprimod Encapsulated PLA on Epithelial Mesencymal Transition in MDA-MB-231 Breast Cancer Cells via STAT3/5 Signaling

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Breast cancer (BC), is the most common malignancy among women worldwide with more than 1 million cases annually. One-fourth of the BC is basal-like triple-negative BC (TNBC) which is the most aggressive and prevailing BC type with a high recurrence rate, risk of metastasis, and overall short survival. The molecular machinery underlying TNBC chemoresistance is identified as surface markers, hypoxic factors, drug efflux transporters, and signaling pathways such as JAK/STAT3. One of the azaspiran family members that acts as an effective STAT3 and STAT5 inhibitor is atiprimod. The apoptotic effect of atiprimod on hepatocellular carcinoma, multiple myeloma, acute myeloid leukemia, and BC cells regarding various cellular processes such as immunosuppressive, ER stress, and autophagy was established. Our aim in this study is to assign the potential incline in atiprimod anti-proliferative effect *via* polylactic acid (PLA)- capsulation in MDA-MB-231 cells through Epithelial-Mesenchymal Transition (EMT). Atiprimod was encapsulated to the PLA polymer using the single emulsion evaporation method. Atiprimod-loaded PLA particles were characterized by scanning electron microscopy (SEM), FTI-R, Zetasizer, and HPLC methods. PLA-capsulated atiprimod effectively decreased MDA-MB-231 cell viability loss in time- (0-72 h) and dose-(0,5-4 µM) dependent manner by MTT cell viability assay. PLA capsulation increased the growth inhibition of Atiprimod in MDA-MB-231 BC cells. Moreover, PLA-Atiprimod treatment increased the colony and migration inhibition of MDA-MB-231 BC cells. When we checked the expression profile of EMT markers by RT-PCR, epithelial markers (E-cadherin) expression increased, and mesenchymal marker (vimentin, N-cadherin) was downregulated following 72 h PLA-capsulated atiprimod in MDA-MB-231 BCs.
This project was supported by the Marmara University Scientific Research Commission (BAPKO) with the project number FDK-2023-10893.

Keywords: Atiprimod, Polylactic Acid PLA, Encapsulation, STAT3, STAT5, Epithelial-Mesenchymal Transition

P017
Investigating ULBP1 and CD155 Expression in Lung Cancer: Insights into NK Cell-Mediated Immunity

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Background/Aim: Lung cancer's high mortality underscores the urgent need for effective treatments. NK cells, pivotal in cancer immunity, utilize diverse receptors for cytotoxicity against cancer cells. Natural killer cell group 2 receptor D (NKG2D) and DNAX-associated molecule-1 (DNAM-1), are two key activating NK cell receptors involved in tumor recognition. UL-16 binding protein 1 (ULBP1), a ligand for NKG2D, is recognized as a prognostic biomarker in various cancers, whereas CD155 is a significant ligand for DNAM-1, playing a critical role in NK cell-mediated metastasis suppression. Based on these insights, this study aims to explore ULBP1 and CD155 expression across diverse lung cancer subtypes, potentially identifying new targets with therapeutic potential.

Materials and Methods: Total RNA was isolated from DMS114 (small cell lung cancer (SCLC) cells), A549 (non-small cell lung cancer (NSCLC) cells), and MRC5 (control) cell lines using the QIAGEN-RNeasy Mini Kit. Subsequently, RNA purity determination, cDNA synthesis and RT-PCR were performed. ULBP1 and CD155 expression levels were quantified using RT-PCR on the Light-Cycler 480 (Roche), with β-actin serving as the reference gene. The $2^{-\Delta\Delta CT}$ method was used to calculate relative changes in gene expression.

Results: The total RNA was purely obtained (260/280: ~2). RT-PCR analysis revealed significant increases in ULBP1 expression in DMS114 (17.5-fold) and A549 (2.3-fold) cells compared to MRC5 controls. CD155 expression showed upregulation in DMS114 (1.36-fold) and A549 (2.5-fold) cells relative to controls.

Conclusion: These findings suggest that ULBP1 and CD155 could serve as valuable biomarkers and therapeutic targets, particularly for SCLC and NSCLC, respectively. Further studies are warranted to validate these targets and explore their potential in enhancing NK cell-mediated cytotoxicity for lung cancer treatment.

Keywords: Lung cancer, ULBP1, CD155, NK cell ligands

P018
Microbial Community Analysis of Van-Erçek Lake,Isolation of Extreme Alkaliphiles from the Lake, Molecular Characterization,Biotechnological Potentials

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Aim: The aim of this study was to elucidate the biodiversity and characterize alkaliphilic microorganisms isolated from the soda-rich environment of Van Erçek Lake. The characterization of these isolates and the determination of their potential to produce specific enzymes were examined.

Materials and Methods:Water samples were collected, and pure culture isolations were performed. The identification of the isolates was conducted using molecular methods. In addition to culture-dependent studies, molecular techniques such as PCR, DNA sequencing, total DNA extraction, Fluorescence In Situ Hybridization (FISH), Denaturing Gradient Gel Electrophoresis (DGGE), and Amplified Ribosomal DNA Restriction Analysis (ARDRA) of the 16S rRNA gene were used.

Results: Ten different isolates from the sampling region were compared with reference sequences using the BLAST program in NCBI for their 16S rRNA gene sequences. BLAST results revealed, for the first time, the presence of *Halobacillus, Paracoccus, Micrococcus, Halomonas, Halopseudomonas, Arsukibacterium, Virgibacillus, Marinomonas, and Pseudomonas* genera in Erçek Lake. According to the results of the metagenome analysis, the samples mainly belonged to the Cyanobacteriota phylum. The biotechnological potential of the isolated genera for producing amylase and protease enzymes was demonstrated. FISH results indicated that Bacterial members were dominant in the samples.

Conclusion: The isolated bacteria can play a significant role in various fields such as biofuel production, environmentally friendly technologies, and sustainability efforts in the future. They possess substantial biotechnological potential to benefit the environment, agriculture, medicine, energy, and many other areas. To fully harness this potential, advanced research and technological developments are required.

Keywords: Alkaliphiles, Erçek lake, PCR, FISH, DGGE

P019
Effects of Laurus nobilis Seed Extract on Tumor Suppressor TP53 and PTEN Genes in Glioblastoma and Adipose-Derived Mesenchymal Stem Cells

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Laurus nobilis, a member of the Lauraceae family, is cultivated as a spice, medicinal, and ornamental plant in Mediterranean countries and worldwide. Many studies show that L. nobilis leaves and seeds are involved in various biological activities. Extracts obtained from the leaves and seeds of L. nobilis have been reported to cause antioxidant, anti-carcinogenic, apoptotic, and proliferative effects. In this study, the effect of L. nobilis seed extract on the expression of tumor suppressor genes (TP53 and PTEN) in a human glioblastoma cell line (T98G) and adipose-derived mesenchymal stem cells (ADMSC) was investigated. In this context, powdered L. nobilis seeds were dissolved in dimethyl sulfoxide and filtered. T98G and ADMSCs were treated with different concentrations (2.5 to 200 µg/mL) of the L. nobilis solution. MTT (3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used for cell viability analysis, and AO (acridine orange) /PI (propidium iodide) staining was used to show cell death. According to the MTT analysis and AO/PI staining results, two different appropriate doses were determined, and the cells were incubated for 24, 48, and 72 hours. Following incubation, cells were collected for real-time quantitative PCR (qRT-PCR) to examine the expression of TP53 and PTEN genes. According to qRT-PCR results, L. nobilis seed extract upregulated the TP53 gene in both cell types, particularly at 48 hours. Additionally, the PTEN gene was observed to be upregulated in the T98G cell line and downregulated in ADMSCs. Based on this, it was concluded that L. nobilis seed extract can regulate the expression of tumor suppressor genes in different cell types and may be considered a potential anticancer agent.

Keywords: Glioblastoma cell line, Adipose-derived mesenchymal stem cells, Tumor suppressor genes, Laurus nobilis

P020
Innovative Strategies for Breast Cancer: Inhibiting IRE1 RNase activity and Modulating miRNAs in Triple-Negative and Receptor-Positive Breast Cancer

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Background/Aim: Triple-negative breast cancer (TNBC), lacking receptor expression, is highly aggressive, contrasting with receptor-positive types (ER, PR, HER2) resistant to hormonal therapies. Stress disrupts ER protein folding, activating Unfolded Protein Response (UPR) crucial for therapy, specifically through IRE1's kinase/RNase activities cleaving mRNA under ER stress, impacting miRNA-mediated gene expression in breast cancer. This study aims to identify miRNAs regulated by IRE1-RNAse activity in different breast cancer subtypes.

Materials and Methods:MDA-MB-231, MDA-MB-468 TNBC, and T47D receptor-positive cells were cultured in DMEM with 10% FBS, L-glutamine, and antibiotics (37°C, 5% CO2). MCF10A cells were cultured similarly with growth factors, treated with 20 µM MKC8866 (IRE1 RNase inhibitor) for 72 hours. q-RT PCR-based miRNome sequencing in MDA-MB-231, T47D, and MCF10A cells revealed altered miRNA profiles linked to IRE1-RNase. Validated miRNA expression changes and target gene/pathway analysis used online databases. In vitro functional assays employed miRNA mimics/inhibitors, assessing miRNA-3'UTR binding via luciferase assays.

Results: Inhibiting IRE1 RNase activity altered miRNA expression in cancer progression. Using Real-Time PCR-based miRNome arrays, we identified 152 significantly different miRNAs (log2 fold change > 2) in DMSO vs. 20 µM MKC8866-treated MDA-MB-231 cells, 216 in MCF10A, and 37 in T47D cells. Focused on TNBC, we studied downregulated miRNAs (hsa-miR-32-5p, hsa-miR-200c-3p, hsa-let-7f-5p) affecting cancer genes (FRS2, PIK3CA, RPS6KB1) with miRNA mimics. Upregulated miRNAs (hsa-miR-362-5p, hsa-miR-646) were studied with miRNA inhibitors for miRNA-3'UTR mRNA (CDK6, CCDN1) relationships via q-RT PCR. Functional assays highlighted miR-32-5p and miR-200c roles in TNBC gene regulation crucial for survival and proliferation.

Conclusion: The findings indicate IRE1-RNase activity-dependent miRNAs specific to TNBC, suggesting that these miRNAs may regulate key cancer-specific genes that could serve as potential biomarkers for TNBC diagnosis and treatment. Further in vivo studies are warranted to validate these miRNAs as therapeutic targets in TNBC management.
This study was supported by TÜBİTAK Project No. 120Z719

Keywords: Breast cancer, Endoplasmic Reticulum Stress, miRNA, IRE1 RNase activity

P021
Innovative Combinatorial Therapy of Tamoxifen and Benzofuran-Linked Chalcones in Breast Cancer Management

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Introduction and Aim: Breast cancer remains the most prevalent malignancy among women worldwide. Despite the extensive use of various chemotherapeutic agents, the pursuit of more effective treatment strategies is critical. Tamoxifen, an estrogen receptor inhibitor, is widely employed in breast cancer therapy; however, resistance and limited efficacy pose significant challenges. Recent research has highlighted the potential of chalcone derivatives for their anticancer properties. This study aims to evaluate the anticancer activity of a novel therapeutic approach combining tamoxifen with 2-benzofuran-linked chalcone derivatives (complexes 1 and 2) in MCF-7 breast cancer cells.

Materials and Methods: Firstly, the cytotoxicity of tamoxifen-chalcone derivatives combination on MCF-7 and healthy MCF10-A cells was assessed via SRB cell viability assay. To determine apoptosis,triple fluorescent staining and gene expression analyses were performed. The effect on cell migration and invasion was analyzed using cell matrigel migration and invasion assays respectively. The ability of cells to form colonies after combination treatment was tested via colony formation assay.

Results: Results showed that the tamoxifen-chalcone derivatives combination inhibited cell growth in MCF-7 cells in a dose- and time-dependent manner. Triple fluorescent staining and gene expression studies indicated that the combination induced apoptosis. Migration and invasion studies revealed that the combination therapy significantly inhibited cellular migration and invasion. Additionally, the colony formation assay confirmed that the combination treatment suppressed the cell's ability to form colonies.

Conclusion: In summary, the study demonstrated the substantial cytotoxic and apoptotic potential of the tamoxifen-chalcone derivatives combination in breast cancer cells. This combination therapy might be a promising candidate for the treatment of breast cancer.
This study was supported by TÜBİTAK (Project No:222Z274).

Keywords:
Breast cancer, Chalcones, Cytotoxicity, Benzofuran, Tamoxifen

P022
DNA BARCODING FOR THE IDENTIFICATION OF VERBASCUM L. FROM TURKEY

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Background/Aim: The world faces a biodiversity crisis due to rapidly growing industrialization and environmental degradation leading to loss of genetic resources through species extinction. It is crucial to preserve the genetic resources by accurate species identification. Turkey, with three biogeographical zones, is a center of endemism and plant diversity including the *Verbascum* L. genus which has a narrow distribution range, facing significant habitat destruction. Due to morphological similarities, the classification of *Verbascum* species is challenging, and adopting DNA barcoding which uses short and conserved DNA sequences with sufficient variations is of great value. The objective of this study is to use DNA barcoding for the molecular identification of key species of *Verbascum* genus from Turkey.

Materials and Methods: The key Anatolian endemic species of the *Verbascum* genus were provided from the herbarium collection of Nezahat Gökyiğit Botanik Bahçesi and the total DNA was extracted from herbarium leaf samples. Internal Transcribed Spacer (*ITS*), ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit (*rbcL*), and maturase K (*matK*) barcode regions were amplified by PCR and sequenced via Sanger and Next Generation Sequencing. The barcode sequences were used for phylogenetic analysis using MEGA11 according to the CBOL (Consortium of Barcode of Life) standards.

Results: *ITS* and *matK* barcode regions were used in the phylogenetic analysis of 11 *Verbascum* species since the quality of the *rbcL* barcode sequences was insufficient to incorporate into the phylogenetic analysis. Both
ITS and *matK* barcodes were successful in differentiating *Verbascum* species; however, the two-loci analysis showed consistent branching suggesting that *matK* has a higher discriminatory power than *ITS* for the *Verbascum* genus.

Conclusion: The phylogenetic trees constructed overlap with established taxonomic classifications. Further comparisons will be made with the addition of new key species. This study will be a milestone for the molecular-level correction of potential taxonomic issues and contribute to the preservation of *Verbascum* L. germplasm.

Keywords: Verbascum L, DNA Barcoding, Biodiversity

P023
Spectral biophysical cytometry with smart probes: high-throughput and multi-parametric cell phenotyping in health and diseases

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Aim: Cells undergo biophysical remodeling as a response to environmental changes, for survival and to execute their function. Changes in the biophysical properties of cells, such as membrane order, tension, and potential, are crucial to various diseases and have considerable potential as a diagnostic or prognostic marker. Over the years, numerous instruments, such as atomic force microscopy, micropipette aspiration, and optical traps have been developed to investigate the biophysical characteristics of cells. However, these technologies suffer from low throughput, which is a major obstacle to applying them to medical problems that require measuring thousands of cells. Therefore, there is still a major gap in our understanding of how collective biophysical properties of the cells alter during both physiological and pathological processes.

Methods: We introduce a method, spectral biophysical cytometry (SBC), that combines spectral flow cytometry with environment-sensitive probes, enabling high-throughput and multi-parametric biophysical analysis with single-cell resolution. We use SBC to measure the biophysical properties of model membranes, cell lines, and human blood samples.

Results: We demonstrate how biophysical phenotyping accurately distinguishes healthy individuals from patients with atherosclerosis and to assess the severity of disease progression.

Conclusion: This fast, robust, and sensitive method may aid the detection of different cellular pathogenic alterations directly from blood. Furthermore, our technology can be implemented with real-time cell sorting based on multi-parametric biophysical properties. Thus, our technique has the potential to be widely used in a variety of biological, biotechnological, and medical fields, where biophysical properties can be exploited as complementary to current protein or nucleic acid markers to diagnose and treat diseases.

Keywords: High-throughput, Biophysical mapping, Membrane fluidity, PBMCs, Atherosclerosis

P024
Developing a Bioinformatics Workflow to Identify Potential MicroRNAs Targeting Tubulin Tyrosine Ligase

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Background: Microtubules are the most dynamic filaments of cytoskeleton, composing of alpha and beta tubulin proteins. Several post-translational modifications occur on alpha tubulin proteins, including tyrosination. Tyrosination is catalyzed by tubulin tyrosine ligase (TTL) after microtubule depolymerization and it affects microtubule stability thereby several cellular processes. We previously showed an upregulation in TTL protein level in a model of Spinal Muscular Atrophy (SMA). TTL expression was also shown to be misregulated in several types of cancer and a neurodegenerative disease. However, molecular reasons underlying these alterations are unknown. MicroRNAs (miRNAs) are short non-coding RNAs with a length of approximately 22 nucleotides. Dysregulation of miRNAs has been shown to be associated with many cancer types and neurodegenerative disorders. Therefore, in this study, we focused on miRNAs and generated a workflow to identify candidate miRNAs having potential to regulate *TTL* gene expression for further in vitro studies.

Material and methods: To determine candidate miRNAs, first, lists of predicted miRNAs were retrieved from several databases, including TargetScan, miRWalk and miRNet and commonly found miRNAs were identified. The identified miRNAs were then evaluated based on sequence conservation, tissue-specific expression levels, and involvement in pathways, along with the gene ontology terms resulting from functional enrichment analysis.

Results:It was determined that 175 miRNAs for humans and 117 miRNAs for mice are common across different databases. By following this workflow, the number of miRNAs was reduced step by step, ultimately selecting 3 candidate miRNAs for future in vitro analyses.
Conclusion: To understand TTL misregulation in SMA, candidate miRNAs will be analyzed in an in vitro disease model. Since no miRNA has been shown to regulate TTL gene expression, the findings of this study will also help to elucidate the post-transcriptional regulation of TTL and its expressional alterations in other diseases.

Keywords: tubuline tyrosine ligase, microRNA, SMA, alpha tubulin

P025
The Role of mir-21-5p, mir-181b1-5p and mir-155-5p Expression Profiles In EGFR-Aptamer-Mediated Apoptotic Cell Death in MDA-MB-231 Breast Cancer Cells

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Breast Cancer (BC) is the most frequently diagnosed cancer and the second leading cause of cancer-related death among women worldwide. Overexpression of Epidermal Growth Factor Receptor (EGFR) in primary BCs, Triple Negative BC (TNBC) assumed as EGFR-mediated therapy regimens. Aptamers, small ssDNA or RNA oligonucleotides with high binding affinity against target molecules and major specificity. microRNA (miRNA), short noncoding RNAs, are estimated as a key regulator for fundamental cellular signaling pathways and commonly recruit in carcinogenesis. miR-141-3p and miR-181b1-5p are significantly increased in TNBC tissue samples, associated with worse overall survival in BC patients. Increased expression of miR-520b-3p promotes the stemness of BC and the potential to bind EGFR mRNA. Herein, we aimed to evaluate the molecular machinery of EGFR-aptamer triggered apoptotic cell death regarding miRNA expression profiles in MDA-MB-231 BC. EGFR-ssDNA aptamer decreased cell viability loss in a dose- (0-1000 nM) and time (0-72 h)-dependent manner in MDA-MB-231 cell by MTT assay. 200 nM EGFR ssDNA aptamer leads cytostatic effect, preventing the colony formation and migration in MDA-MB-231 BC. EGFR-Aptamer triggered mitochondrial membrane potential loss, cell death, and ROS generation within 72 h aptamer exposure. When we checked the expression profile of miRNA in MDA-MB-231 BC cells by qRT-PCR, miR-21-5p, miR-181b-5p, and miR-155-5p expression levels 4.5, 6, 1.3-fold downregulated following ssDNA aptamer against EGFR, respectively. Significant downregulation in anti-apoptotic proteins (Bcl2, BclxL), Caspase-3, and PAPR cleavage was determined after 72 h EGFR-aptamer (200 nM) treatment in MDA-MB-231 TNBC cell line. Thus, ssDNA aptamer against EGFR established an apoptotic effect on MDA-MB-231 BC cells via acting on miR-21-5p, miR-181b-5p, and miR-155-5p expression profiles.
This project was supported by the Marmara University Scientific Research Commission (BAPKO) with the project number FYL-2023-10996.

Keywords: Triple Negative Breast Cancer, Epidermal Growth Factor Receptor, Aptamer, microRNA

P026
Lysosomal Biophysical Heterogeneity is Disrupted in Niemann-Pick Disease Type C

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Background/Aim:Niemann-Pick disease type C (NPC) is a rare neurodegenerative disorder caused by mutations in the intracellular lipid transporters NPC1 and NPC2 that result in cholesterol and sphingolipid accumulation in the lysosome. The subsequent pathogenic cascade responsible for NPC symptoms is not fully understood. We hypothesise that disrupted lipid transport would alter the biophysical properties of cellular membranes, which may have pathological implications.
Materials and Methods: To measure organelle membrane fluidity of donor primary fibroblasts, we used novel organelle targeting probes, that change their emission spectrum in response to membrane fluidity. To study lysosomes with single organelle resolution, we established advanced imaging methods such as lattice light sheet. Furthermore, we used spectral flow cytometry to obtain high-throughput measurements at the single-cell level. Lastly, we performed next-generation RNA sequencing to understand the cellular response to impaired cholesterol transport.

Results: Our results revealed the emergence of biophysically distinct lysosomal populations in NPC, while plasma and ER membrane fluidity remained unchanged. In addition, lysosome populations showed heterogeneous distribution among NPC cells. Importantly, the clinically approved glucosylceramide synthase inhibitors miglustat and eliglustat only partially corrected the lysosomal biophysical phenotype, while dietary lipid depletion had drastic effects on the NPC lysosomal populations. Last, transcriptomic analyses revealed upregulation of genes involved in
*de novo*cholesterol synthesis, including the key rate-limiting enzyme *HMGCR*.

Conclusion: Our newly established methodologies unveiled the previously unknown biophysical heterogeneity of lysosomes and its disruption in NPC. Despite having lower cholesterol accessibility, cells maintain plasma membrane fluidity through upregulating cholesterol biosynthesis. Treatment with miglustat partially corrected the NPC phenotype by specifically affecting the low fluidity lysosomal populations. However, complete reversion of the lysosomal biophysical state might require novel combinatorial treatments. Importantly, our methodologies can be broadly applied and allow researchers to perform high throughput/resolution characterisation of organelle membrane biophysics in health and disease.

Keywords: Niemann-Pick disease type C, cellular biophysics, organelle membrane fluidity, lysosomes, advanced imaging

P027
Determination of the Effect of Diclofenac and Combined Treatments on Glucose Metabolism in Breast Cancer

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Background/Aim: Breast cancer remains the leading cause of cancer-related deaths among women worldwide. Therefore, there is a need for the development of new treatment strategies. This study aimed to investigate the effect of Diclofenac, which is used clinically as a pain reliever and antipyretic, both alone and in combination with chemotherapeutic agents Docetaxel (Doc) and 5-Fluorouracil (5-FU), on glycolysis in MCF-7 breast cancer cells. Glycolysis is a metabolic pathway that supports the high energy production and growth of cancer cells and is a significant target in anti-cancer research.

Material and Method: For this purpose, initially, viability analyses were performed using the Sulforhodamine B (SRB) method after 48-hour treatments of MCF-7 cells with Diclofenac, Diclofenac+Doc, and Diclofenac+5-FU. After determining the cytotoxic effects, glycolysis was assessed by measuring glucose uptake and lactate release, which are the main indicators of glycolytic activity in the cells.

Results: The results showed that combination treatments caused a significant reduction in MCF-7 cell viability compared to treatments with Diclofenac, Doc, and 5-FU alone. While Diclofenac alone did not cause a significant change in glucose uptake and lactate release, glycolytic activity was inhibited after the combination treatments.

Conclusion: These data suggest that Diclofenac, when used in combination with standard chemotherapeutic agents like Doc and 5-FU, not only increases cytotoxic effects but also disrupts the glucose metabolism of breast cancer cells. Our study highlights the potential of using Diclofenac in combination with chemotherapeutic drugs to achieve more effective anti-cancer strategies through metabolic modulation.

This study was supported by Bursa Uludag University Scientific Research Projects Unit (project number THIZ-2023-1735).

Keywords: Diclofenac, Warburg Effect, Breast Cancer, Combination Therapy

P029
Anti-cancer Potential of Postbiotics Derived-from Ligilactobacillus animalis Against Breast Cancer

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Background / Aim: Breast cancer is considered a significant global health problem, globally. Since conventional treatment strategies cause side effects, novel and effective treatment methods are still required. At this point, the anti-cancer potential of microbial postbiotics attracts wide attention. This study aims to investigate the anti-breast cancer effectiveness of postbiotics derived from *Ligilactobacillus animalis*.

Materials and Methods: Postbiotics were obtained following the cultivation of the strain. Their selective and effective cytotoxic dose on breast cancer cells (MCF-7) was determined by MTT assay after 48 h co-treatment. Flow cytometry analysis was performed to determine the effects of postbiotics on the apoptosis and cell cycle. The effects of the postbiotics on clonogenic activity, cell motility, and intracellular reactive oxygen species (ROS) accumulation of MCF-7 cells were also evaluated. Furthermore, expression levels of genes related to
anti-cancer mechanisms were analyzed by quantitative Real-Time PCR.

Results: The concentration of 1.500 µg/mL of postbiotics which did not display any cytotoxic effects on the healthy cells but significantly reduced the viability of the MCF-7 cells, was selected for further analysis. Inhibition of cell proliferation was also confirmed by decreased clonogenic activity and cell motility. Moreover, postbiotics induced apoptosis as evidenced by subG1 accumulation, an increase in annexin V, and apoptotic gene activation in MCF-7 cells. Furthermore, postbiotic treatment leads to a significant increase in intracellular ROS levels of MCF-7 cells and an up-regulation of the genes related to endoplasmic reticulum stress-mediated apoptosis.

Conclusion: The results of the current study indicate that postbiotics can be used as an adjuvant approach to cancer treatments.

Keywords: Postbiotics, Breast Cancer, Apoptosis, Cell Cycle, ER Stress

P030
Exploring Mutations and Transcriptomic Changes in Scoliotic Zebrafish through Comprehensive Data Driven Multi-Omics Analysis Pipeline

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Background/Aim: Adolescent idiopathic scoliosis, a progressive spinal deformity affecting ~4% of the population, has an underlying pathogenesis that remains poorly understood due to its complex genetics. Limited human tissue-specific genetic studies have led zebrafish to become a powerful model for studying the genetic basis of human scoliosis. Here, we developed a multi-model sequencing data analysis pipeline using the publically available data from scoliotic zebrafish to identify the key genetic variants and pathways involved in the development of scoliosis.

Materials and Methods: We integrated publicly available whole-genome, whole-exome, and RNA-Seq data from wild-type and scospondin-hypomorpic mutants to reveal the genetic variations of scoliosis. Candidate variants were predicted by isolating variations unique to the mutants through multi-model data integration and assessed with principal component (PCA) and differential gene expression (DEG) analysis. The DEGs were subjected to network and enrichment analysis to identify their biological functions.

Results: Our pipeline discovered 16 variants in 30dpf fish, coinciding with the age showing the highest variation in PC1. Tg and Lgals were directly associated with the 30dpf-scoliosis phenotype. Within the whole data, ENSDARG00000095518, a novel gene of unknown functionality, displays the highest degree of betweenness and eigenvector centrality, highlighting it as a promising candidate for further studies. Syndig1 and Dusp14 also demonstrated significant centralities, underscoring their roles in scoliosis. Thoracic vertebral growth was linked to overexpression of Syndig1l in the brain and cerebellum, while Dusp14 was associated with skeletal abnormalities, including scoliosis, validating the accuracy of our novel analysis pipeline.

Conclusion: Our multi-modal omics pipeline revealed that the pathophysiology of zebrafish idiopathic scoliosis is significantly influenced by genetic variants in Syndig1 and Dusp14, which are involved in synaptic transmission and skeletal development, and ENSDARG00000095518, of unknown functionality. Future research, including the modulation of these candidate genes, holds promise for discovering new therapeutic targets for scoliosis treatment.

Keywords: Idiopathic Scoliosis, Zebrafish Model, Genetic Variants, Transcriptomes, Differential Gene Expression

P031
Isolation and Characterization of Prostate Cancer Cell-Derived Exosomes for Drug Delivery

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Background/Aim: Exosomes are extracellular vesicles, 30-120 nm in size, secreted from many cell types and function in intercellular signal transmission. Since their structure is compatible with the cell membrane, exosomal drug carrier systems have significant potential in the efficient delivery of therapeutics. Further, cancer cell-derived exosomes (CCD-exo) are advantageous in targeting tumor cells in comparison to the ones from other cell types because of their homing ability to reach the tumor site of origin. Thus, CCD-exo can passively target cancer cells and the tumor microenvironment supporting personalized cancer treatment strategies. This study aims to isolate and characterize exosomes from various prostate cancer cell lines for their further use in drug delivery for prostate cancer treatment.

Materials and Methods: Exosomes from the LNCaP, PC-3, and DU145 cells were isolated by ultracentrifugation method. For the characterization of the obtained exosomes, size and zeta potential were measured with dynamic light scattering. Exosomes were visualized by STEM confirming their size and morphology. Then, the exosomal markers such as CD9, CD63, CD81, HSP70, and HSP90, were investigated by western blot.

Results: Exosomes derived from prostate cancer cell lines were determined to be 80-120 nm in size. In addition, STEM analysis confirmed their round spherical-like morphology. Relatively high CD9 expression was detected in exosomes derived from all prostate cancer cell lines while CD63 expression was undetectable. Further, CD81, HSP70, and HSP90 expressions, which were relatively high in CCD-exos were detected to be distinctly expressed in exosomes of PCa cell lines.

Conclusion: Results indicated that the isolation and characterization of prostate cancer cell-derived exosomes exhibited the necessary standards such as size, morphology, and exosomal protein markers. Our data may be a valuable source and provide a standard for further research on utilizing prostate CCD-exos as drug delivery systems in the treatment of prostate cancer.

Keywords: prostate cancer, exosomes, exosomal delivery system, cancer cell-derived exosomes

P032
A Multiparametric and High-Throughput Platform for Host–Virus Binding Screens

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Background/Aim: Initial binding of virus particles with their surface receptors in the host cell are appealing antiviral drug targets to prevent virus entry and subsequent infections. However, there are two main roadblocks that hinder the investigation of the underlying mechanisms of initial virus-host interactions at the plasma membrane. First, structural complexity of virus and host cell surfaces complicate the study of individual molecular actors due to their complex interplay. Second, the small size of viral particles well below the diffraction-limit of light makes them inaccessible to several methods and techniques.
Materials and Methods: In this work, we designed a defined and adjustable bottom-up membrane model to enable high-throughput drug screening of virus-host interactions on multiple scales. Forming a synthetic membrane on silica beads allowed subsequent attachment of recombinant His-tag modified host receptors, mimicking a minimal host cell with controllable compositions. Using this system, we investigated host-virus interactions in more detail using qualitative confocal microscopy and quantitative lattice light-sheet microscopy. Moreover, attachment of the small SARS-CoV-2-mimicking virus-like particles to the synthetic host cells allowed us to perform quantitative high-throughput flow cytometry.

Results: Using these complementary techniques we screened the blocking-efficiency of different nanobodies and human serum samples for viral particle binding events. Moreover, besides the binding of SARS-CoV-2 like particles to the potential host cell proteins ACE2, TMPRSS2, CD26, CD147, and CD304 we also investigated their binding to the sialylated glycosphingolipid GM1.

Conclusion: In summary, we present a fast and easy-to-prepare defined model membrane system which enables the investigation of virus-host interactions on multiple scales. The platform will facilitate and speed up the search for specific drugs and represent a valuable technique for pandemic preparedness.

Keywords: host-virus interactions, model membranes, membrane biophysics

P033
Investigation of Interactions of Baloxavir with Selected Influenza Polymerase Binding Sites by Molecular Modeling

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Background/Aim: Influenza A viruses (IAVs) are a major health concern in terms of causing annual seasonal epidemics, which resulted in a noteworthy number of cases and 290,000 to 650,000 deaths each year (WHO, 2023). Although different antiviral drugs are widely used for the treatment of IAVs, genetic changes related to antiviral resistance may occur due to high mutation rates in the viral genome. In fact, despite the administration of the recent antiviral drug, Baloxavir Marboxil (BXA) have been started on 2018 (CDC, 2022), substitutions (I38T, E23K) on the polymerase acidic (PA) protein have been identified (Omoto et al., 2018). Although several studies have focused on I38T substitution in terms of BXA resistance, the molecular basis remained limited. Therefore, to enable deeper understanding on the antiviral resistance at the atomic level, this study focused on the interactions between BXA and selected amino acids using quantum mechanical methods.

Materials and Methods: To achieve this goal, the conformer analysis was performed with Spartan 14 (v1.1.4) with molecular mechanics tool of MMFF (Merck Molecular Force Field). Geometry optimizations and frequency analyses were conducted using density functional theory (DFT) at □B97x-D/6-31G+(d,p) level using Gaussian 16 software.

Results: As a result of conformer distribution analysis of BXA, Isoleucine, Threonine, Glutamic Acid, Lysine, Alanine and Tyrozine initial conformers were obtained. Based on the energies the most stable structures were selected. The most stable conformers of amino acids and BXA interaction will be investigated. Non-covalent interactions will be visualized and compared to each other.

Conclusion: This study will be elucidated the importance of non-covalent interactions in terms of binding properties of different amino acids to BXA. The well-known BXA resistance marker mutations on PA protein, I38T and E23K, will be investigated. As a result of this study the most susceptible bonds and interactions of BXA regarding antiviral resistance will be detected.

Keywords: Molecular Modelling, Antivirals, Influenza A, Baloxavir

P034
Suramin Has Antioxidant and Anti-Inflammatory Effects on the Small Intestine in a Mouse Colitis Model

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Background/Aim: Dextran sulfate sodium (DSS) is often preferred to induce an ulcerative colitis model in experimental animals. DSS affects the small intestine as well as the colon. Suramin, used as a therapeutic agent in African trypanosomiasis, is a histone deacetylase inhibitor that can inhibit NAD^+-dependent histone deacetylases and exhibits broad biological effects, including antiproliferative and antiviral activities. In our study, we aimed to investigate the antioxidant and anti-inflammatory effects of suramin on the intestinal injury caused by DSS in mice.

Materials and Methods: In our study, 32 C57BL/6 mice were divided into four groups. DSS group mice were administered 3% DSS dissolved in drinking water for five days. Suramin was administered intraperitoneally at a dose of 25 mg/kg for seven days. Malondialdehyde (MDA) and glutathione (GSH) levels, as well as myeloperoxidase (MPO), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities, were determined spectrophotometrically in intestinal tissues. Expression levels of cyclooxygenase-2 (COX-2) and proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), were determined by western blotting.

Results: DSS application caused oxidative damage in the intestinal tissues by increasing MDA levels and MPO activity, and decreasing GSH levels and CAT, SOD, and GPx activity. Additionally, DSS stimulated inflammation by increasing COX-2, TNF- α, and IL-6 levels. Injection of suramin into DSS-given animals significantly increased in intestinal GSH levels and antioxidant enzyme activities while reducing the elevated MDA levels. Suramin prevented the intestinal injury in the DSS-induced colitis model by decreasing COX-2, TNF- α, and IL-6 expression levels.

Conclusion: This study showed that suramin has antioxidant and anti-inflammatory effects on the small intestine in a mouse colitis model. The protective effects of suramin observed intestinal tissue suggest that it may be a useful therapeutic agent for inflammatory bowel diseases.

Keywords: suramin, dextran sulphate sodium, ulcerative colitis, small intestine, mice

P035
Bacilysin and Sporulation: Rescue of Bacilysin Mutant Phenotypes by Sporulation Transcription Factors

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Background/Aim: Bacilysin is a dipeptide antibiotic produced by *Bacillus subtilis*. Previous studies have shown that when bacilysin production is disabled, *B. subtilis* produces defective endospores with lowered resistance, defective germination and altered sporulating colony pigmentation. Through previous mass spectrometry and transcriptomic studies, we have shown that such mutants also have lowered expression levels of sporulation related genes, including the five key sporulation transcription factors RsfA, SpoIIID, GerR, SpoVT and GerE. The goal of this study is to observe if the mutant phenotypes are caused by the lack of these transcription factors, and whether their overexpression through multicopy plasmids would rescue the bacilysin mutant sporulation phenotypes.

Materials and Methods: The five genes of interest were cloned into the *E. coli*/*B. subtilis* shuttle vector pHT08 and transformed into the bacilysin KO *B. subtilis* strain OGU1. The following phenotypes were compared between OGU1, OGU1 overexpressing the transcription factors, the wild-type strain PY79, and mutant strains lacking the expression of the transcription factors: spore count, spore resistances to wet heat, lysozyme and chloroform, germination kinetics and colony pigmentation.

Results: The results show that no single transcription factor can rescue all mutant phenotypes, but some can rescue single phenotypes. Chloroform resistance was rescued by SpoIIID, while lysozyme resistance was rescued by SpoIIID, SpoVT and RsfA. GerE and GerR improved the spore count of OGU1. All five proteins improved the germination rate of OGU1. GerE rescued the mutant colony pigmentation of OGU1.

Conclusion: The sporulation defects found in OGU1 can be partially rescued through the overexpression of the five sporulation transcription factors RsfA, SpoIIID, GerR, SpoVT and GerE, though one alone cannot rescue all defects. It appears that the phenotypes of bacilysin negative strains are not caused by the lack of a single transcription factor, but rather a lack of them all.

Keywords: Bacillus subtilis, Bacilysin, Sporulation, Transcription factors, Microbiology

P036
Bacilysin and Sporulation: Regulation of Bacilysin Production by Sporulation Transcription Factors

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Background/Aim: Bacilysin is a dipeptide antibiotic produced by *Bacillus subtilis*. Previous studies have shown through electrophoretic mobility shift assays (EMSAs), beta-galactosidase assays and bioassays that genes involved in the initiation of sporulation such as CodY, AbrB, Spo0A and ComA control *bac* expression. The goal of the study is to observe whether the sporulation transcription factors RsfA, SpoIIID, GerR, SpoVT and GerE regulate the expression of the *bac* operon.

Materials and Methods: The five transcription factors were cloned into the *E. coli*/*B. subtilis* shuttle vector pHT08 and transformed into the bacilysin KO *B. subtilis* strain OGU1, and the effect of their overexpression on *bac* operon expression were studied through the *bac*/*lac* fusion via beta-galactosidase assay. They were also cloned into the *E. coli* plasmid pET28a(+), overexpressed, and purified using IMAC columns and used in EMSAs to observe *in vitro* DNA binding between them and the *bac* promoter. Supernatants obtained from *B. subtilis* strains mutant in the genes of interest were used in bioassays to observe changes in bacilysin overexpression caused by the mutations.

Results:EMSA results show direct DNA binding between SpoIIID and GerE and the *bac* promoter. Beta-galactosidase results show that GerR and RsfA overexpression increase *bac* expression while SpoVT, SpoIIID and GerE overexpression lower it. Bioassay results show small reductions of bacilysin in SpoIIID and GerE mutants, and significant reductions in GerR and SpoVT mutants.

Conclusion: SpoIIID and GerE have been shown to directly bind the *bac* promoter, though beta-galactosidase and bioassay results have shown they have little effect on expression. SpoVT couldn't bind DNA *in vitro*, however it has been shown to be important for *bac* expression in its bioassay, though the beta-galactosidase assay have conflicting results. GerR appears to be an important factor in bacilysin regulation, increasing expression in the beta-galactosidase assay and significantly lowering expression when absent in the bioassay.

Keywords: Bacillus subtilis, Bacilysin, Sporulation, Gene regulation, Microbiology

P037
Investigation of the Effects of Mutations in the FYR Module of Kabuki Syndrome-Associated KMT2D Gene on Protein Interactions and Chromatin Organizatio

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Background/Aim: Kabuki Syndrome is a rare disease characterized by various developmental and neurological disorders, affecting approximately 1 in 32,000 individuals. Mutations in the *KMT2D* gene are found in 55-80% of patients. This project aims to investigate the effects of different types of mutations in the N-terminus and C-terminus of the FYR domain of KMT2D on protein-protein interactions and chromatin organization.

Materials and Methods: We modeled the effects of two specific mutations on the protein structure using structural biology approaches with the AlphaFold tool. The R5179C mutation is a missense mutation in the N-terminus part of the FYR domain, while the R5282* mutation results in a truncated protein at the C-terminal end. Wild type and mutant KMT2D proteins will be overexpressed in HEK293T cells and changes in protein interactions caused by the two mutations will be determined using the RIME (Rapid Immunoprecipitation Mass Spectrometry of Endogenous Proteins) method. Additionally, the effects of these mutations on chromatin accessibility will be determined using the ATAC-seq method. Subsequently, ATAC-seq will be also performed using PBMCs obtained from the blood of a healthy volunteer and a Kabuki Syndrome-patient with the R5282* mutation. Finally, all results will be comprehensively analyzed and correlated with the patient's clinical data.

Results: Plasmids containing the three forms of KMT2D have been created and overexpressed in HEK293T cells. ATAC-seq and RIME protocols have been applied to these cells, and the analysis results are pending. Structural modeling of the two mutations, both separately and together, has been completed using AlphaFold, demonstrating that the mutated forms can be reliably modeled similar to the wild type.

Conclusion: There are currently no therapies available for the Kabuki Syndrome other than symptomatic treatments. It is anticipated that this project will identify new molecular targets, which will enable development of specific treatment strategies for Kabuki Syndrome.

Keywords: Kabuki Syndrome, KMT2D, protein-protein interactions, FYR domain

P038
Turkish and Korean Sweetpotato [Ipomoea batatas (L.) Lam] Cultivars: Screening for Plant Viruses and Molecular Identification via DNA Barcoding

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Background/Aim: The sweetpotato is a nutritious crop gaining recognition as a superfood. However, its vegetative propagation makes it susceptible to viral diseases, contributing to low production levels of local varieties in Turkey. To protect and develop local varieties for commercial production, it is crucial to cleanse plants of viral diseases using molecular virus screening techniques, ensuring crop health and productivity. Furthermore, molecular identification of plants is essential for conserving genetic resources, asserting rights, and genetic fingerprinting.
This study aims to detect common plant viruses in Turkish and South Korean sweetpotato cultivars, and molecularly identify and asses the evolutionary differences via DNA barcoding.

Materials and Methods: Three Turkish and six South Korean sweetpotato cultivars were screened for Sweetpotato Chlorotic Stunt Virus (SPCSV), Sweetpotato Feathery Motile Virus (SPFMV), Sweetpotato Leaf Curl Virus (SPLCV), Sweetpotato Virus C (SPVC) and Cucumber Mosaic Virus (CMV) using PCR with virus-specific primers.Turkish cultivars were molecularly identified using *matK*, *ITS* and *rbcL* DNA barcodes and compared to South Korean cutivars. Barcode regions were amplified via PCR and sequenced via Sanger sequencing. The consensus sequences were used for phylogenetic analysis using MEGA11 according to the CBOL (Consortium of Barcode of Life) standards.

Results: All tested viruses were present in Turkish sweetpotato cultivars, while SPLCV and CMV were detected in South Korean cultivars. *matK*, *ITS* and *rbcL* barcode regions were used in phylogenetic analysis of sweetpotato cultivars. Single locus analysis showed the *matK* region had the least discriminatory power. Two-loci (*matK* and *ITS*) and three-loci analyses revealed consistent branching in maximum likelihood trees, indicating the *ITS* region had the highest discriminatory power in sweetpotatoes.

Conclusion: This study provided a foundation for pathogen screening to obtain virus-free plant material. Phylogenetic analysis revealed that Turkish and South Korean sweetpotato cultivars diverged into separate branches.

Keywords: Ipomoea batatas, Sweetpotato, Molecular virus screening, DNA barcoding

P039
Prediction of Binding Pockets: The Dynamic Gateways of Protein Function

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Background/Aim: Proteins frequently interact with other molecules, including other proteins, small molecules, or DNA/RNA, through binding pockets on their surface. These dynamic gateways enable proteins to undergo structural and dynamic changes upon binding, triggering a cascade of effects on protein function. Thus, it is essential to consider protein internal motions to understand the dynamics of protein binding pockets and predict their locations. This study presents a novel approach for predicting protein binding residues by integrating protein dynamic behavior, from global to local dynamics, with geometric evaluations.

Materials and Methods: We employed the Gaussian Network Model (GNM) to dissect residue fluctuations across the dynamic spectrum, from the slowest to the fastest modes of motion. Our focus was on the extremes: slow modes, which reveal flexibility, and fast modes, which indicate stability. By analyzing the intersection of slow-mode hinges and high mean-square distance fluctuations of fast-mode residues with FPocket-identified cavities, we aimed to uncover a link between stability, flexibility, and binding residues, ultimately developing a protocol to identify functional binding pockets.

Results: We tested our approach on a dataset of 50 proteins (monomers and multimers) with distinct functions. The initial results showed a sensitivity of 21.2%, specificity of 97.2%, precision of 17.4%, and accuracy of 94.8% in predicting known binding sites. Lower sensitivity values indicate that not all the binding pocket residues are dynamically important, while lower precision points to new functionally plausible binding pockets.
Conclusion: The findings highlight that protein binding pockets are integral to the dynamic infrastructure of proteins. It offers valuable insights into binding interactions within the context of the protein's overall dynamics, hinting at their role in allostery and changes in protein shape and function.

Keywords: Protein Dynamics, Binding Pockets, Protein-Ligand Interactions, Gaussian Network Model

P040
Combined Delivery of P53 Activating and KRAS Inhibitory Small RNA Significantly Inhibited Cell Proliferation and Motility In Human Lung Cancer Cell

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Background/Aim: Small RNAs are used in many studies, including the investigation of cancer treatments, to modulate gene expression both in terms of activation and inhibition. For our study that aimed to reveal the anticancer effect of the combined use of siRNA, which inhibits KRAS expression, and saRNA, which activates p53 expression, in cancer cells carrying mutant KRAS and wild-type p53, compared to their separate transfections, and to develop a new RNA-based therapeutic approach.

Materials and Methods: In the study, A549 human lung adenocarcinoma cell line was used as a model. The cells were transfected with small RNAs by using gold nanoparticules. Post-transfection mRNA and protein expression analyzes were performed by RT-qPCR and Western Blot methods, respectively. The events at the cellular level caused by transfection were examined by performing cell viability test, cell cycle analysis, apoptosis and necrosis test, colony formation experiment, wound healing experiment, invasion and migration experiments. The results were evaluated statistically by applying Student's t-test.

Results: Co-transfection of small RNAs at a concentration of 50 nM was found to result in a significant increase in p53 mRNA levels ($p<0.001$) and protein levels ($p<0.001$), and did not alter KRAS mRNA and protein levels ($p>0.05$) in the A549 cell line, compared to their individual transfections. It was determined that the combined treatment approach more effectively arrested the cell cycle at the G0/G1 phase, triggered apoptosis to a greater extent, and significantly reduced cell proliferation, colony formation ability, invasion, and migration compared to individual transfections.

Conclusion: It has been demonstrated that the combined approach subject to our study provides an anticancer effect for human lung cancer cell line compared to the control group, and that this approach has a much greater anticancer effect than the anticancer effect provided by each of the small RNAs separately.

Keywords: p53, saRNA, KRAS, siRNA, A549

P041
EGF Promotes Neuroendocrine-Like Differentiation of Bladder Cancer

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Aim: Bladder cancer (BC) is one of the most common and deathly malignant diseases. Nearly 90% of BC originates from urothelium. Urothelial carcinoma is histopathologically divided into 2 classes: Muscle invasive bladder cancer (MIBC) and non-muscle invasive bladder cancer (NMIBC). Neuroendocrine-like bladder cancer (NE-like BC) is one of the subtypes of MIBC. Although NE-like BC has a very poor prognosis, information on the molecular characterization of these tumors is very limited and there are not yet any models available mimicking neuroendocrine differentiation in BC. The aim of this project is to model neuroendocrine differentiation in bladder cancer via EGF treatment.
Materials and methods: MIBC cell lines were induced with Epidermal Growth factor (EGF). The phenotype of the cells was characterized using bright field and confocal microscopy. NE-marker and epithelial-to-mesenchymal transition (EMT) signature profiles were analyzed with qRT-PCR and Western Blot. The status of Akt-Erk activation was identified by Western Blot. Nuclear localization of β-catenin was determined using immunofluorescence (IF) staining.

Results: Phenotypically, we identified that EGF stimulated neurite-like extensions in BC cells, which was validated by phalloidin staining. Also, the expression of neuroendocrine marker genes such as *ENO2* and
ChgA was significantly increased. Additionally, our results revealed that EGF treatment resulted in a significant reduction in cell proliferation rates, and disruption in cell-to-cell interaction, indicating the induction of epithelial-to-mesenchymal transition (EMT). This finding was confirmed by down-regulation of E-cadherin at both mRNA and protein levels. We also demonstrated that EGF treatment increased nuclear translocation of β-catenin in MIBC cell lines. Next, we showed that Akt-Erk pathway, which is a downstream effector of EGF Receptor (EGFR) signaling, is activated in BC cell lines upon EGF treatment.

Conclusion: Our findings suggest that EGF stimulates neuroendocrine differentiation in bladder cancer, and thorough understanding of this process may provide a therapeutic target option for neuroendocrine-like bladder cancer.

Keywords: Bladder Cancer, EGF, Neuroendocrine differentiation

P042
Gene Delivery with Use of Transferrin Receptor Targeted Cationizable Lipo-Oligoaminoamide

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Background/Aim: The therapy with nucleic acids showed great potential to cure life-threatening, severe diseases, especially cancer. Non-viral gene delivery is a promising alternative to the classical method of viral gene delivery. However, non-viral nanocarriers need to be improved in several aspects such as suitable size, high stability outside of the cells, low affinity to blood components, high affinity to target cells, efficient uptake, successful endosomal escape, and finally disassembly inside the cells. This study focuses on the transferrin receptor-targeted plasmid DNA (pDNA) delivery with previously designed sequence-defined T-shaped lipo-Oligoaminoamides (OAAs).

Materials and Methods: Only 12 amino acid length protease-resistant synthetic retro-enantio transferrin receptor (reTfR) ligand was used as a targeting agent. Six lipo-OAAs differing in their lipidic domains and redox-sensitive attachments of lipid residues were synthesized and tested in order to evaluate the impact of core polyplex stability on receptor-dependent gene transfer. Biophysical characterization of pDNA nanoparticles were investigated via DLS, ELS, TEM, agarose gel shift and ethidium bromide exclusion assays. *In vitro*
biological characterizations were done with luciferase gene transfer assay, cell viability assay, cellular association and competition assays.

Results: The reTfR-targeted pDNA polyplexes demonstrated receptor-targeted gene delivery in TfR-rich K562 and N2a cells with a small advantage when using the bivalent DBCO-containing conjugate reTfR-2 compared to monovalent conjugate reTfR-1. Interestingly, each cell line showed different lipo-OAAs to be the best for transfection. The less stabilized NonOcA-OAA 1258 or 1285/reTfR-2 was found to be suitable for pDNA delivery into erythroleukemic K562 cells and the ssbb-based bioreducible DecA- or NonOcA-OAAs 1284 or 1285/reTfR-2 was best suited for neuroblastoma N2a cells. The blockade of TfR by preincubation with iron-saturated hTf reduced the cellular association of polyplexes which supports the hypothesis of a TfR-mediated gene transfer process of these reTfR polyplexes.
Conclusion: Overall, transferrin receptor-targeted sequence defined T-shaped lipo-OAA based specialized non-viral nanocarriers have been generated and used for pDNA transfer successfully.

Keywords: Nanotechnology, Gene Therapy, Non-viral vectors, Nanoparticles

P043
Redesign of SARS-CoV-2 Nanobodies for COVID-19 Treatment

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Background: Severe acute respiratory syndrome coronavirus (SARS-CoV-2) is a global health crisis with increasing number of infections. Receptor-binding domain (RBD) of SARS-CoV-2 Spike protein binds to angiotensin-converting enzyme 2 (ACE2) receptor on the host cell for the viral entry. Since this interaction is critical for the therapeutic targeting of COVID-19, it is important to inhibit this interaction using various small or large molecules. Our research, in this line, aims to design novel peptides that can get integrated to the existing nanobodies to improve the target binding affinity.

Materials and methods: We found peptides that target RBD-ACE2 interaction using *in silico* studies. After synthesizing these peptides, we studied their binding affinity for ACE2. Among these four peptides, we focused on one of them and integrated its gene sequence to TY1 nanobody on its CDR3. After transformation to *E. coli* BL21 cells, its bacterial production was optimized, and total protein was isolated from periplasmic space with further purification to over 90% purity. Its secondary structure was determined using Circular Dichroism (CD), showing mostly β-sheet structure. *In-vitro* binding experiment was performed with protein of interest and RBD, revealing moderate amount of binding.

Results: Our results showed that there is interaction between peptide-integrated nanobody and RBD, however, our preliminary results from immunoblotting analysis testing *in-vitro* binding led us to speculate that the nanobody's binding affinity to RBD was greater than that of the peptide-integrated nanobody. Further experiments to test binding kinetics are necessary for a better conclusion.

Conclusion: It is important to find better blockers for RBD-ACE2 interaction, therefore, we plan to investigate peptide sequences that can enhance the binding affinity for ACE2 using nanobodies.

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Keywords: SARS-CoV-2, nanobody, virus neutralization, peptide

P044
Endogenous Tagging of Iroquois Complex Genes Reveals Unique Expression Patterns for Araucan, Caupolican and Mirror in Drosophila

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Background/Aim: The Iroquois Complex (Iro-C) genes code for evolutionarily conserved proteins crucial for the development of sensory systems. In Drosophila, these genes include araucan (ara), caupolican (caup) and mirror (mirr). They are involved in the regulation of eye development and the regulation of rhodopsin and olfactory receptors genes. ara, caup and mirr are located in the same genomic locus and show high homology, which complicates the characterization of their individual roles in development using classic approaches such as the GAL4-UAS system. Here, we present the endogenous tagging of Iro-C genes and characterize and compare their individual expression patterns.

Materials and Methods: Plasmids for CRISPR/Cas9 gene editing of the Iro-C genes were cloned, and lines expressing ara::HA::GFP, caup::V5::GFP, and mirr::FLAG::GFP were generated. Tagging was verified by PCR and sequencing, and expression patterns of Iro-C genes were visualized using IHC.

Results: In the eye-antennal disc, the expression of ara and caup reaches the highest level in the dorsal part of the eye territory of the disc, near the dorsoventral boundary, and in the most anterior region of the antennal territory. The expression pattern of mirr partially overlaps with ara and caup and is tightly focused in the most dorsal region of the eye territory and in the anterior and posterior regions of the antennal territory of the disc. In the wing disc, all Iro-C genes are expressed in the notum and hinge territories of the disc, on the periphery of the wing territory, and only ara and caup are expressed in the center of the wing pouch.

Conclusion: The expression pattern of mirr differs from ara and caup.

This work was supported by TUBITAK grant no 215Z369 and Bogazici University Research Fund BAP17B01M6 to A.C.

Keywords: Iroquois, Drosophila melanogaster, CRISPRCas9

P045
Revealing The Structure Of The Native Turkish Origanum L. Collection At The Aegean Agricultural Research Institute (AARI)

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Origanum L. (oregano) is one of the most commonly used and studied medicinal plant species from the family Lamiaceae. Oregano grows naturally in the Mediterranean region with many endemic species that have diverged in distinct areas. Hybridization events throughout the history of this genus are known to be the main cause of oregano's speciation. In this work, *Origanum* germplasm consisting of specimens collected in Türkiye and maintained and cultivated at the AARI Institute was screened with ten polymorphic Sequence Related Amplified Polymorphism (SRAP) markers. The average gene diversity (GD) was observed as 0.32 for the collection, while the highest GD was 0.41 for SRAP marker em4me3. Neighbor Joining dendrogram analysis indicated that the collection fell into four clusters. However, analysis of population structure using a Bayesian approach revealed two main sub populations reflecting common hybridization events resulted in an admixed genetic structure. This finding was also supported by the Analysis of Molecular Variance results which indicated high within population diversity in the collection indicating a decrease in the exclusiveness of species between sections. According to the results, the most common hybridizations occurred between sections: *Origanum* and *Majorana*, and between the pair of species: *Origanum vulgare* subsp. *hirtum* and *Origanum syriacum* subsp. *bevanii* and *Origanum onites* and *Origanum syriacum* subsp. *bevanii*. Our findings suggest that SRAP markers can be utilized to understand hybridization dynamics of oregano species collected from their natural habitats. Moreover results from such analysis can be used to target certain specimens or sections for further breeding and conservation management purposes.

Keywords: oregano, genetic diversity, hybridization, speciation

P046
Discovery of bacterial secondary metabolites in boron mine tailing ponds using shotgun metagenomics

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Background/aim: Tailing ponds are part of mining facilities that contain waste material from mining. Boron mine tailing ponds possess high boron and pH concentrations that promote the proliferation of bacteria adapted to extreme pond conditions. Bacteria inhabited in tailing ponds produce a variety of bacterial secondary metabolites encoded by biosynthetic gene clusters (BGCs). BGCs are co-located genes that corporately work to produce metabolites such as toxins, pigments, and antibiotics. The discovery of novel BGCs is important for medicine and biotechnology. However, many bacterial BGCs remain unidentified, especially in bacteria living in extreme conditions. In this study, we applied shotgun metagenomics and bioinformatics analysis to discover novel BGCs in boron tailing ponds.

Materials and methods: The biological materials were obtained from the boron tailing ponds in Eskişehir. The water samples were passed through a polycarbonate filter, and the total genomic DNA was isolated. The DNA library was prepared using the QIAseq FX DNA Library Kit and sequenced on the Illumina NovaSeq 6000 system. The reads were pre-processed with RQCFilter. FastQC was used to check the quality of reads. MEGAHIT was used to assemble reads, and the quality of assembly was checked with QUAST. antiSMASH was used with all extra features to identify the secondary metabolites for the assembly reads.

Results: A total of 44 BGCs were identified in assembly reads. We found that there were 19 types of BGCs. The terpene type is the most common in our dataset, followed by NRPS, RIPP-like, betalactone, and hserlactone.

Conclusion: Our study suggests that the boron mine tailing pond could be a valuable biological source of novel bacterial secondary metabolites.

Keywords: Boron, shotgun metagenomics, bacterial secondary metabolites, bioinformatics, tailing ponds

P047
Uncovering bacterial diversity in boron tailing ponds through metagenome-assembled genomes

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Background/aim: Metagenome-assembled genomes (MAGs) are complete or near-complete genomes reconstructed from shotgun metagenomics sequencing acquired from environmental samples. Boron tailing ponds are repositories for waste materials from boron mining and present an extreme environment for microbial life due to the high boron and pH concentrations. Despite these extreme conditions, a diverse range of bacterial species inhabits tailing ponds. The metagenomic approach allows to assembly of MAGs to identify and characterize bacterial species at the genomic level. In this work, we performed shotgun metagenomics sequencing of the sample collected from boron tailing ponds and constructed MAGs to identify bacterial composition.

Materials and methods: The biological materials were obtained from the boron tailing ponds in Eskişehir. The total genomic DNA was isolated and sequenced on the Illumina NovaSeq 6000 system. RQCFilter was used to remove contaminating reads. FastQC was used to check the quality of reads. MEGAHIT was used to assemble shotgun metagenomics sequencing reads. DAS-Tool was used to obtain optimized consensus bins from MaxBin2, MetaBAT2, and CONCOCT. CheckM was used to assess genome qualities. GTDB-Tk was used for taxonomic assignments of bacterial genomes.
Results: We identified a total of six high-quality MAGs (completeness ≥90, contamination ≤5) belonging to Bacteroidia, Cyanobacteria, Gammaproteobacteria, and Alphaproteobacteria. The five MAGs were taxonomically classified as *Aquiflexum lacus, Nodosilinea antarctica, Belliella aquatica, Oceanibaculum nanhaiense,* and *Roseicitreum antarcticum,* and one of the MAGs remained unclassified at species levels.

Conclusion: Our analysis suggests that shotgun metagenomics provides valuable insights into the bacterial diversity in boron tailing ponds. Our data also indicate that unclassified MAGs at the species level demonstrate the presence of potentially novel bacterial taxa in boron tailing ponds.

Keywords: Metagenome-assembled genomes, shotgun metagenomics, bioinformatics, boron, tailing ponds

P048
Development of A Human Cell-based Prion Blood-Brain Barrier Chip Model

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Background: Prion diseases, a group of rare and incurable neurodegenerative disorders, include familial forms in humans. Mutations in the *PRNP* gene lead to the conversion of native cellular prion protein (PrP
[c]) into β-sheet-rich, protease-resistant misfolded prion protein (PrP^{Sc}), contributing to these disorders. This transformation results in neuropathological manifestations, occasionally accompanied by spongiosis, and amyloid plaques formation. However, potential alterations in the blood-brain-barrier (BBB), particularly within the brain endothelial cells due to the pathogenesis, have not been reported yet. Conventional 2D cell culture methods fall short in replicating *in vivo* brain microenvironmental factors, and due to variations in protein profiling among species, animal models cannot fully emulate human physiological or pathological conditions. Consequently, there is an urgent need to establish an *in vitro* prion model based on human cells, providing physiologically relevant cellular microenvironment. This study aims to comprehend the impact of prion on the
BBB due to prion by developing a novel human cell-based Prion BBB-on-a-chip.

Method: Brain-like endothelial cells (BLECs) are differentiated from human induced pluripotent stem cells (hiPSCs) obtained from both prion patients and healthy individual. These cells are then co-cultured with human primary astrocytes and pericytes in a two-channel microfluidic device to establish an *in vivo*-like BBB microenvironment. Assessment of barrier integrity and functionality in healthy versus diseased BBB microenvironments involves utilizing multiple techniques such as trans-endothelial electrical resistance (TEER) measurements, permeability assay, and immunostaining to detect endothelial tight junction and adherence proteins.

Result: hiPSC-derived BLECs exhibit different expression levels of tight junction markers. Additionally, the prion BBB-on-a-Chip model has revealed changes in BBB permeability, indicating potential issues with BBB integrity and functionality.

Conclusion: This innovative human cell-based prion BBB-on-a-Chip model represents a significant advancement in the study of blood-brain barrier function, providing a robust platform to explore physiological and pathological interactions within the brain microenvironment.

Keywords: Prion, blood-brain-barrier BBB, human induced pluripotent stem cells

P049
Inflammation-Induced Reactive Stroma Alters EMT and Cell Adhesion Markers in Epithelial-Myofibroblast Co-Culture Model of Prostate Cancer

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Background/Aim: Co-culture and 3D cell culture models that represent the complex tumor microenvironment and interactions of different cell populations are valuable tools for creating in vitro disease models and investigating novel treatment strategies/drug candidates. Therefore, this study aims to establish a co-culture model of prostate cancer to investigate the role of tumor-stroma interaction in cellular adhesion and migration of prostate cancer cells stimulated by inflammatory microenvironment.

Materials and Methods: LNCaP, DU145, and PC3 cancer cells were co-cultured with WPMY1 myofibroblasts in a transwell system by using an inflammatory conditioned medium containing TNF-α and TGF-β1. Reactive stroma, epithelial mesenchymal transition (EMT), and cell adhesion markers were analyzed by western blot. Scratch, anoikis, and transwell migration assays were performed on prostate cancer cells. 3D prostate cancer cell spheroids were generated in poly-HEMA-coated U-bottom 96-well plates to further investigate the EMT-related alteration due to inflammation by western blot.

Results: Epithelial-myofibroblast co-culture model of prostate cancer demonstrated that the inflammatory microenvironment induces a-SMA expression in fibroblasts leading to reactive stroma. However, androgen-dependent and castration-resistant prostate cancer cells showed distinct responses to inflammation-induced stromal effects. Altered expression of fibronectin, vimentin, ICAM1, EpCAM, and HCAM due to inflammation in epithelial cancer cells under co-culture conditions suggested that stimulated stroma supports mesenchymal-like morphology in the epithelial-mesenchymal transition of the tumor epithelium altering cellular adhesion and enhancing tumor cell migration. In addition, EMT factors such as Twist-1, Snail-1, fibronectin, and NKX3.1 were regulated differently in prostate cancer spheroids treated with an inflammatory medium when compared to monolayer cultured cells.

Conclusion: An epithelial-myofibroblast co-culture model was established to better mimic the tumor-stroma interaction in inflammatory tumor microenvironment and our data revealed that reactive stroma supports inflammation-related tumorigenic changes. This study demonstrates that co-culture and 3D cell culture techniques provide valuable data for disease progression and therapeutic strategies for prostate cancer.

Keywords: Co-culture, 3D cell culture, Prostate Cancer, Inflammatory Tumor Microenvironment, Tumor-stroma Interaction

P050
Phenotypic Comparison of Epithelial Cells from Thymus and Umbilical Cords

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Background/Aim: The thymus is a primary lymphoid organ for T lymphocyte development and maturation. Absence of functional T cells results in severe immune deficiency. Thymic epithelial and stromal cells play an important role in the maturation of T cells, but lack of sufficient thymus material makes it difficult to generate functional thymus organoids. The umbilical cord (UC) stroma contains both epithelial and stromal stem cells. To assess the potential use of these cells to generate thymus organoids, we compared thymus and UC epithelial cells.
Materials and Methods: Thymic cells were isolated from pediatric donors. Thymi were washed with PBS, minced and run through 40 um cell strainers to obtain a single cell suspension. UCs were rinsed with PBS, the amnionic membrane was removed and cut in small pieces and cultured in M-171 medium with supplement. Cells were grown and expanded until passage 3. Total thymic stromal cells and umbilical cord epithelial cells were assessed for presence of the cell surface markers CD29, CD31, CD44, CD54, CD73, CD90, CD105, CD200 and HLA-DR.

Results: The thymic epithelial and stromal cell mixture expressed high levels of CD29, CD31 and CD44 (>70%), medium expression of HLA-DR (50%) and showed low expression of the mesenchymal markers CD73, CD90 and CD105 and epithelial markers CD54 and CD200 (<3%). In contrast, UC epithelial cells showed high expression of CD29, CD44, CD73 and CD105 (>90%), medium expression of CD54 and CD200 (>50%), and low expression of CD31 and HLA-DR.

Conclusion: Analysis showed the presence of mixed stromal populations in whole, thymic cell preparations, consisting of endothelial, epithelial and stromal cells, whereas culture of UC epithelial cells resulted in the isolation of CD54 and CD200 expressing epithelial cells, that also co-express mesenchymal cell markers. Further purification of thymic cell populations will allow better comparison of the epithelial cell contents.

Keywords: Thymic cells, Umbilical cord epithelial cells, Mesenchymal stem cells

P051
Anastasis Mechanism in Non-Small Cell Lung Cancer Cells and Relationship with Metastasis

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Background: This study investigates anastasis, the reversal of apoptosis, in Non-Small Cell Lung Cancer **(**NSCLC) cells, elucidating its implications for metastasis. Anastasis challenges conventional apoptosis understanding by illustrating that cells can return from a proapoptotic state upon removal of the apoptotic stimulus. This phenomenon is profoundly significant as it demonstrates that cancer cells can escape programmed cell death and contribute to tumor survival, drug resistance and metastasis.

Materials and Methods: A549-csp cell lines, which express caspase-3 biosensors, were established in A549 cells. Paclitaxel (PTX) was utilized to induce apoptosis, and the maximum apoptotic response was assessed at different time intervals. PTX treatment, caspase-3 positive cells were isolated using flow cytometry and subsequently cultured without the apoptotic inducer. Gene and protein expression levels associated with apoptosis and metastasis in the returning anastatic cells were analyzed using RT-PCR and western blot techniques. Additionally, pathway analyses and protein interaction networks were investigated using the R Bioconductor “clusterprofiler” package.

Results: The results demonstrate a progressive decrease in caspase-3/7 activity and mitochondrial membrane potential in cells cultured without PTX, indicating a shift towards an anastatic phenotype. Moreover, the decrease in caspase-3/7 and BAX levels, along with the increase in BCL2 levels, suggests that anastatic cells evade apoptotic cell death. Additionally, a reduction in E-cadherin levels, associated with metastasis, and an increase in N-cadherin levels, a marker of the metastatic process, were observed. Elevated expression of metastasis-related proteins such as Snail, Slug, and Fibronectin further supports these findings. Finally, bioinformatics-based protein network analyses indicate the activation of the metastatic cascade following cessation of the apoptotic process.
Conclusion: This study represents the first in vitro validation of the anastasis phenomenon in NSCLC cells and demonstrates that this mechanism plays a critical role in understanding the death escape strategies and metastatic potential of cancer cells.

This research was funded by TÜBİTAK 1001 (123Z073) Projects Support Program.

Keywords: Anastasis, non-small cell lung cancer, apoptosis, metastasis

P052
Screening and Functional Characterization of Differentially Expressed Genes in Brain Regeneration

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In order to maintain normal brain functioning and cognitive abilities in response to an injury, brain regeneration is an essential process. Nevertheless, in humans, the capacity for brain regeneration is limited, especially in adults. Conversely, zebrafish have an outstanding ability to regenerate their brains after extensive damage. The focus of this project is the investigation of a gene called Legumain, which is identified via the screening of differentially expressed genes in the zebrafish telencephalon. Expanding on the knowledge acquired from the study named "Comparative Transcriptome Analysis of the Regenerating Zebrafish Telencephalon," our goal is to clarify the spatial and temporal expression patterns of Legumain during traumatic brain injury in adult zebrafish.
Employing in situ hybridization, immunofluorescence assays, and CRISPR-Cas9 knockout, we seek to characterize Legumain's role in brain regeneration and its potential interactions with key pathways. According to in situ hybridization assay, it is established that Legumain expression is increased in injured telencephalic sections compared to the control group. The sections are grouped as 3, 7, and 14 days. In immunofluorescence staining assay, it is shown that Legumain is synthesized in actively dividing cells, rather it is more pronounced at the late differentiation and integration stage (7-14 days) compared to the early proliferative stage (3-7 days).
These findings propose that Legumain has an essential role in adult zebrafish brain regeneration. Completing this project successfully will not only contribute to uncovering novel therapeutic targets for brain injury repair but also offer valuable insights for developing targeted interventions to increase brain regeneration in response to traumatic brain injury.

Keywords: zebrafish, brain regeneration, legumain

P053
Effects of ADAMTS15 and PCDH20 Genetic Activation on Angiogenesis and Metastasis in Melanoma Cells

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Some characteristics of cancer and tissue regeneration are similar, such as cell migration and cell proliferation. Tumors, however, are distinct from regenerated tissue because of their invasive development, and aberrant proliferation. Expanding on earlier studies that interpreted the transcriptome profiles of malignant melanoma and regenerating melanocytes in zebrafish, we discovered that ADAMTS15 (ADAM Metallopeptidase with Thrombospondin Type 1 Motif 15) and PCDH20 (Protocadherin 20) are differently regulated genes in melanoma and regeneration.

The initial purpose of this study is to investigate the functions of the PCDH20 and ADAMTS15 genes in a SKMEL-28 and MeWo melanoma cell line with a focus on their potential role as tumor suppressors. Genetic activation of these target genes separately achieved via CRISPR-Cas9 gene editing with a multiple sgRNA plasmid using the golden-gate assembly cloning technique. Target gene-activated cells with transfection technique are injected into the yolk sac of zebrafish embryos to investigate their impact on malignant features such as angiogenesis and metastasis.

Experiments in the SK-MEL-28 melanoma cell line indicated that cell metastasis was significantly decreased in zebrafish xenografts with ADAMTS15 and PCDH20 genes that were genetically activated. Similarly, angiogenesis assays showed that MeWo cells revealed a notable decrease in angiogenesis where target genes were activated. These findings imply that ADAMTS15 and PCDH20 genes can suppress cancer progression by reducing the angiogenesis level and metastasis rate.

Our study aims to provide insights into the tumor-suppressive functions of ADAMTS15 and PCDH20 genes in melanoma. Differential regulation of signaling in regeneration versus cancer can be harnessed to halt tumor growth and it can offer new anticancer therapies.

Keywords: zebrafish, melanoma, regeneration, metastasis, angiogenesis

P054
Investigation of the Regenerative Potential of Proteoglycan 4a in Mammalian Neuron and Glia Cells

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Proteoglycans (PGs) are heterogeneous groups of glycoproteins found in the extracellular matrix (ECM) and they play roles in the regulation of cellular events such as proliferation, differentiation and cell migration. Proteoglycan 4 or lubricin belongs to the PG group and is a highly glycosylated proteoglycan. It has been shown that the addition of *Prg4* after traumatic brain injury (TBI) reduces neuroinflammation and restores blood-brain barrier function. It has also been observed that *Prg4a* expression increases during the regeneration process after TBI. However, the exact role of *Prg4a* in this process is not yet known. The aim of this study was to determine the role of *Prg4* in mammalian neurons and glial cells after TBI and non-traumatic brain injury (NTBI).

Two different cell lines were used for the experiments: human neuroblastoma cells (SH-SY5Y) and adult mouse neuroglial cells (FNG). *Prg4* expression was investigated in cultured mammalian cells modified with TBI and NTBI. *Prg4* mRNA and protein expression were determined using Western blot and quantitative-real-time PCR (qPCR) techniques.

The normal levels of certain genes in the cell lines were determined by the qPCR method. Changes in the expression of oligodendrocyte markers were examined to confirm the presence of demyelination. The effects of
Prg4 on damage repair will be tested with gain of function in the later stages of the experiments. The effect of
Prg4 on damage repair will be investigated by transfection of *Prg4* plasmid into SH-SY5Y and FNG cells. GFP plasmid will be used as a control. The increase in *Prg4* expression will be confirmed by Western blot analysis and the response of cells to demyelination will be investigated using qPCR and immunoflourescent (IF) staining.
We hope that the contribution of the *Prg4* gene to the regeneration process after TBI will be more clearly understood with the results of these experiments.

Keywords: zebrafish, traumatic brain injury, non-traumatic brain injury, regeneration, demyelination

P055
Investigation of the Therapeutic Potential of Humanin-Loaded Nanoparticles in Alzheimer's Disease in a Zebrafish Amyloid β42 Toxicity Model

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Alzheimer's disease (AD) is characterized by amyloid-β (Aβ) plaques and phosphorylated tau tangles which eventually cause cognitive decline, affect daily life activities and reduces the quality of life. Since the current therapies for AD are symptomatic, new therapy methods are needed to stop or slow down the disease. Humanin is a neuroprotective agent and Gly14-humanin is 1000 times efficient humanin variant. So that, in this project the effect of Gly14-humanin on AD biomarkers in Aβ42 toxicity induced zebrafish AD model will be tested.
Gly14-humanin packed with solid lipid nanoparticles (SLN) targeted with transferrin (Tf) and polyethylene glycol (Peg) to facilitate the passage across the blood-brain barrier (BBB) by using retro-orbital (RO) microinjection technique. By injecting Aβ42 into adult zebrafish brain through the cerebroventricular microinjection technique, AD model will be created. Changes in the expression of AD-related genes, apoptosis, synaptic degeneration, altered protein expression will be examined after microinjection of the Tf-humanin, Peg-humanin and Tf-Peg-humanin. Techniques like immunofluorescence staining, confocal fluorescence microscopy, western blotting, quantitative real-time PCR and bioinformatics analyses like proteomic will be used.
50 nmol, 100 nmol, 200 nmol, 400 nmol concentrations of SLN, Tf-SLN, Peg-SLN, Tf-Peg-SLN formulations applied to zebrafish embryo environment and embryo development is observed 24 and 48 hours later under stereomicroscope and 100 nmol is selected as nontoxic concentration for formulations. 2 µl of 100 nmol formulations are injected into adult zebrafish brain by RO and images of zebrafish brains are compared among fluorescence intensity and find out that Tf-Peg-SLN crosses the BBB more efficiently.
Further studies will focus on investigating Gly14-humanin packed Tf-Peg-SLN to cross the BBB and the effects on the toxicity of Alzheimer's disease in AD model. According to the outcome of these experiments, therapeutic potential of humanin-loaded nanoparticles in Alzheimer's disease will be evaluated.

Keywords: zebrafish, humanin, Alzheimers disease

P056
Investigation of T and B Cell Subsets in Pediatric Eosinophilic Esophagitis Patients in the Turkish Population

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Background / Aim: Eosinophilic esophagitis (EoE) is a chronic allergic inflammatory condition of the esophagus that is triggered by specific food and/or environmental allergens. It is characterized by T helper 2 (Th2)-driven immune response. The immune response observed in EoE differs between adults and pediatric patients and elevated circulating T regulatory (Treg) cell levels in pediatric patients is one such difference. There is not enough study that covers the EoE-related immune response seen in Turkish pediatric EoE patients. In this study, we investigated circulating T and B cell subsets in Turkish pediatric EoE patients compared to their non-EoE controls.

Materials and Methods: Patients in this study were aged between 2-17 and were confirmed to be EoE by both clinical data and eosinophil counts within the tissue. We isolated peripheral blood mononuclear cells (PBMCs) to perform flow cytometry stainings to analyze T and B cell subsets. For intracellular and intranuclear stainings, we stimulated cells with phorbol myristate acetate (PMA)/Ionomycin with Brefeldin A for 5 hours. Data were analyzed using FlowJo 10.8.1 Software and GraphPad Prism 8.0.

Results: Our preliminary data showed that TIGIT+ T helper (Th) cells were significantly higher in peripheral blood of pediatric EoE patients compared to their non-EoE controls. Additionally, we observed a non-significant increase in the levels of T follicular helper (Tfh), IL-10+ Th, and IL-4+ Th cells.

Conclusion: Our data suggests that the levels of certain T and B cell subsets differ between pediatric EoE and control groups, yet patient numbers should be increased. We will perform a polymerase chain reaction (PCR) assay for EoE-related genes for our follow-up studies. This project is funded by Istanbul Technical University, Department of General Research Projects (ITU-GAP) (Project ID: 43425) and Scientific and Technological Research Council of Turkey (TUBITAK-1002B) (Project ID: 124S482).

Keywords: EoE, Pediatric, T cell, B cell

P057
Mitochondrial Complex I inhibitor rotenone affects Helicobacter-activated B cell survival and differentiation

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Background/Aim: Mitochondrial energy metabolism plays a crucial role in immune cell survival, differentiation, and function. Research indicates that inhibiting mitochondrial electron transport system (ETS) Complex I with rotenone reduces IL-10 production in CpG (TLR9 ligand) stimulated B cells. However, the effect of rotenone on *Helicobacter*-activated B cells remains unclear. Therefore, our study, aimed to explore the impact of rotenone on the survival, proliferation, and IL-10 secretion of *Helicobacter felis* (*H. felis*) stimulated B cells.

Materials and methods: We magnetically sorted B cells from the spleen of VertX IL10 ^{egfp} mice and stimulated them with *H. felis* antigen (10 ug/ml), TLR2-ligand PAM3CSK4 (2.5 ug/ml) or TLR4-ligand LPS (5 ug/ml) in the presence or absence of different doses of rotenone (80nM or 160 nM) for 24 and 48 hours. For the proliferation assay, we labeled the cells with CFSE and cultured them for 72 hours. We assessed cell viability and IL-10 production at 24 and 48-hour time points by staining with 7-AAD and analyzing the IL-10 GFP signal using flow cytometry.

Results: Our results show that rotenone significantly reduces B cell viability and IL-10 production, particularly at the 48-hour time point. The viability of stimulated B cells decreased from 80-85% to 50-70% with 80 nM rotenone and to 30-50% with 160 nM rotenone. Proliferation of stimulated cells also dropped from 60% to 20-40% as the rotenone dose increased. While IL-10 production in PAM3CSK4 and *H.felis*-activated B cells dramatically decreased, there were no changes in IL-10 production in LPS-activated B cells.

Conclusion: Our findings indicate that mitochondrial energy metabolism is essential for the expansion and IL-10 production of *Helicobacter*-activated B cells. Further studies are needed to investigate the impact of rotenone-treated, *Helicobacter*-stimulated B cells on $CD4^+$ T cells. This study was supported by the TUBITAK. (Project Number: 119S447)

Keywords: B cell, Helicobacter, Mitochondria, Rotenone

P058
The Link Between Depression, Sleep, and Synaptic Density in the Elderly

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Background/Aim: Depression in older adults is a prevalent and debilitating condition that significantly impacts quality of life, contributing to increased disability and overall illness burden. With the ageing population growing worldwide, understanding the progression of depression and its interrelationships with other age-related changes is crucial. This study explores the trajectories of depression, its impact on mortality, and its association with sleep disturbances and synaptic density in the ageing brain.

Materials and Methods :Data were used from the University of Manchester Longitudinal Study of Cognition in Normal Healthy Old Age cohort, with 6,375 participants aged 65 and older. Depression trajectories were analyzed using generalized linear mixed models. Mortality risks associated with depression were evaluated using Cox mixed-effects models. Longitudinal associations between sleep duration and depression were examined through generalized structural equation modelling. Post-mortem brain tissue samples were analyzed to assess synaptic density using immunoreactivity measurements of Synaptic Vesicle Glycoprotein 2A (SV2A), focusing on various brain regions, including the frontal lobe.

Results: Depression rates declined until age 80, then increased. Depression was associated with a 10% increased risk of mortality. Reduced sleep duration was significantly correlated with higher levels of depression, and depression predicted subsequent reductions in sleep duration. Post-mortem analysis indicated a global reduction in synaptic density in individuals with depression, with the most pronounced decreases in the frontal lobe, a region that is critical for cognitive function.

Conclusion: Addressing depression in older adults is crucial for improving mental health, reducing mortality risk, and preserving neurobiological health. Early detection and intervention are essential to mitigate adverse effects on sleep, synaptic density, and overall cognitive health. Comprehensive care strategies that integrate mental health, sleep management, and neurological preservation are needed to enhance the well-being and longevity of older adults.

Keywords: Depression, Older adults, Sleep, Synaptic density, Mortality

P059
Discovery and Characterization of Novel CRISPR Effectors

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Background/Aim: CRISPR technology has revolutionized programmable biology, paving the way for curative treatments for various diseases. CRISPR effectors vary in functions, binding preferences, editing efficiencies, and immunogenicity profiles. While some natural orthologs have been identified, further discovery and characterization of novel genome editors are needed.

Materials and Methods: Metagenomic sequences were downloaded from the NCBI dbGaP database. Following quality control, trimming, and assembly, proteins were predicted using Prodigal. CRISPR arrays were identified with Piler-CR, and Hidden Markov Model (HMM) profiles of Cas1 and Cas2 were employed to detect auxiliary proteins. Proteins neighboring CRISPR arrays, larger than 700 amino acids, were BLASTed against the NCBI-NR database. Proteins with high similarity to known effectors were excluded, followed by 3D structural prediction using AlphaFold2 and identification of trans-activating CRISPR RNA.

Results: Analysis of 2.7 TB of data revealed over 80 novel candidates from diverse CRISPR systems. Some candidates appear to cleave double-stranded DNA with blunt ends, while others may produce sticky ends. Three Cas9 orthologs were selected for initial in vitro studies, focusing on characterizing Protospacer Adjacent Motif (PAM) requirements via HT-PAMDA experiments and subsequent functional testing in mammalian cells.

Conclusion: Unlike previous studies, our approach utilizes flexible parameters concerning effector size and the distance between effectors and arrays, providing a more unbiased discovery method. Structural modeling and comparison allowed for a deeper understanding of diversity. We anticipate our project will expand the genome editing toolbox with a range of use cases regarding cleavage mechanisms, delivery size, and immunogenicity.

Keywords: CRISPR, Cas9, Metagenomics, PAM

P060
Investigation of the Neuroprotective Effects of Resveratrol and Hydrogen sulfide in an in vitro Parkinson's Model

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Background/Aim: Parkinson's disease (PD) is the second most prevalent neurodegenerative disease, being characterized by dopaminergic neuronal loss. Polyphenols are group of natural compounds that have been shown to offer protection against various diseases, including PD. Among these, found in several plants, including grapes, blueberries, raspberries, and peanuts, resveratrol (RSV), exhibits neuroprotective effects through its antioxidative capabilities. Hydrogen sulfide (H_2S), one of the main endogenous gaseous transmitter, is implicated a novel neuromodulator against neurotoxin-induced neurodegeneration in PD. The present study aimed to investigate the neuroprotective effects of RSV and H_2S on 6-hydroxydopamine (6-OHDA)-induced SH-SY5Y cells.

Materials and Methods: Cells were pre-treated with RSV and H_2S for 4 h, and then post-treated with 6-OHDA (50 µM) for 24 h. Cell viability (MTT), cytotoxicity (LDH), caspase-3,-8,-9 levels, reactive oxygen species (ROS) levels, and mitochondrial membrane potential, were all evaluated.

Results: The 6-OHDA induction significantly decreased cell viability, increased LDH release, caspase-3, -8 and -9 protein activities, ROS formation, and mitochondrial membrane potential. Combined and pre-treatment with RSV and H_2S increased cell viability, while reducing LDH, caspase-3, -8, -9 levels, ROS levels, and mitochondrial membrane potential.

Conclusion: Our results support the neuroprotective potential of RSV and H_2S at low concentrations against the damaging effects of 6-OHDA and constitutes a promising tool for the development of therapeutic strategies against PD-related neurodegeneration.

Keywords: Parkinsons disease, SH-SY5Y cells, resveratrol, hydrogen sulfide, 6-hydroxydopamine

P061
Generation of Lacrimal Gland Organoids from Mouse Embryonic Stem Cells

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Background/Aim: The lacrimal gland provide to nourish and shield the eye by producing tears, but malfunction of lacrimal gland can lead to severe dry eye disease. Artificial tears, anti-inflammatory drugs, and punctal occlusion all offer short-term fixes but don't address the underlying issue. Limited regenerative ability of adult lacrimal glands need new models for innovative treatments. Organoids are 3 dimension (3D) structures derived from embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult stem cells that can self-organize and resemble the morphology and functions of organs. Due to these capabilities, they are excellent models for studying lacrimal gland biology and treatment. In this study, we aimed to develop lacrimal organoids from mouse embryonic stem cells (mESC).

Material and Method: mESCs were seeded on U-bottom 96-well plates (5000 cell/100 ml) and cultured in ectodermal differentiation medium at 37 °C in a humidified incubator containing 5% CO2 for two days. On the third day of differentiation, non-neural ectoderm was induced by adding 10 ng/mL recombinant bone morphogenetic protein 4 (BMP4) and 1 mM SB431542 to the cultured cells. On the 4rd day, ocular surface ectoderm was induced by adding 10 ng/ml BMP4 and 1 mM IWP2. The cells were cultured for 6 days and the medium was replaced with maturation medium containing 1% Matrigel on day 10. One-half of the medium was replaced with maturation medium without matrigel every other day until day 30.

Result: mESCs appeared as dome-shaped, bright and tightly packed colonies, and it was seen that these cells maintained pluripotency by staining with OCT3/4 and SOX2 antibodies. Budding in lacrimal gland organoids began to appear on the 14th day.

Conclusion: The data obtained from the study will contribute to the understanding of the developmental biology of the human lacrimal gland and provide to test therapeutic treatments for dry eye disease.

Keywords: Lacrimal gland, Organoids, mESC, Dry eye syndrome, Stem cells

P062
Comparison of Different Methods for In Vitro Modelling of Inflammatory Bowel Disease

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Background/Aim: Together Crohn's diseases and Ulcerative colits are known as Inflammatory Bowel Disease (IBD). In IBD due to inflammation induced damage, the intestinal barriers are affected and permeability significantly increases due to opening up of the tight junctions between the cells of the intestinal epithelium. Here, we aimed to develop an *in vitro* model of IBD, that is characterized by increased permeability and loss of intestinal barrier integrity, using DSS or cytokines to induce damage to murine small intestine organoids (enteroids).

Materials and Methods: Small intestines from 8-12 weeks old healthy Balb-c mice were collected and enteroids were isolated and cultured up till passage 2. Enteroids were stained with Chromogranin A for enteroendocrine cells, Mucin 2 to detect goblet cells, and Cleaved Caspase 3 to visualize apoptotic cells. In order to induce an IBD-like environment in enteroids, TNF-a, IFN-ɣ and DSS were administered for 24-48 hours and permeability was assessed using FITC-Dextran and visualized using fluorescence microscopy. Analysis of fluorescence intensity was performed using the Image J program.

Results: Enteroids contained both Chromogranin A expressing enteroendocrine cells and Mucin 2 positive goblet cells. Caspase 3 expression was not observed, indicating the absence of apoptosis in healthy enteroids. Culture of the enteroids in presence of DSS, TNF-a and IFN-ɣ caused an increase in luminal permeability of the enteroids in all treatment groups, indicating induction of cell damage. Especially induction with DSS for 48 hours was highly effective in causing damage and intestinal permeabilization.

Conclusion: Enteroids represent a similar environmental organization as in vivo intestinal crypts, containing stem cells, enteroendocrine and goblet cells. Healthy enteroids are devoid of apoptotic cells, but induction with either DSS, TNF-a or IFN-ɣ can rapidly induce cellular damage and increased permeability, similar to in vivo IBD. Using this model, potential treatments of IBD can be tested in vitro.

Keywords: Organoids, Inflammatory Bowel Disease, Enteroids

P063
Characterization of Fetal Umbilical Cord-derived Stem Cells and Comparison with Adult Bone Marrow MSCs

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Background/Aim: The umbilical cord (UC) contains different types of fetal stem cells, derived from distinct germ layers, that can be easily isolated and expanded without any ethical concerns. Among these, the UC mesenchymal stromal/stem cells (MSCs) are the most well-known and characterized cells. In contrast, few studies have assessed the UC lining epithelial cells (CLECs) for their stemness and differentiation potential. Here, we aimed to characterize fetal tissue-derived UC-MSCs and CLECs to reveal potential differences for their use in regenerative medicine and to compare the data with adult bone marrow (BM) MSCs.

Materials and Methods: The UCs obtained from healthy newborns were used for isolation of UC-MSCs and CLECs and cultured. UC-MSCs, BM-MSCs, and CLECs were assessed using flow cytometry for expression of the hematopoietic markers CD34 and CD45, epithelial marker CD54, MSC markers CD73, CD90, CD105, and immune modulation-related marker CD200. RT-qPCR was performed to assess the expression of pluripotency related genes *hTERT*, *OCT3/4, SOX2,* and *NANOG*, epithelial cell markers *p63* and *CK19*. Normalization was done using *GAPDH*.

Results: Whereas UC-MSCs expressed high levels of CD73, CD90, CD105, and CD200 (>90%) and were negative for the hematopoietic markers CD45 and CD34 (<2%), CLECs expressed high levels of CD73, CD90, and CD105 (>90%), intermediate levels of CD54 and CD200 (>50%). Gene expression analysis indicated that both CLECs and UC-MSCs display a more immature genotype with increased expression of *hTERT, OCT3/4, SOX2,* and *NANOG* in comparison to BM-MSCs. Differences in CK19 and p63 expression between the fetal tissue stem cells were not significant, indicating that overall fetal tissue stem cells show distinct, but overlapping characteristics in both phenotype and genotype, that are clearly distinct from adult tissue stem cells.

Conclusion: Fetal tissue-derived UC-MSCs and CLECs express higher levels of pluripotency genes in comparison to adult BM-MSCs and show overlapping expression of cell surface markers.

Keywords: cord lining epithelial cells, umbilical cord mesenchymal stem cells, bone marrow stem cells, pluripotency

P064
Combination Therapy of Chalcone Complex and VPA: Suppression of Metastasis in Papillary Thyroid Cancer Cells

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Background/Aim: Papillary thyroid carcinoma (PTC) represents a predominant endocrine malignancy, comprising 80-85% of all thyroid carcinomas. The presence of aggressive tumor variants within PTC complicates treatment with surgery or radiation. There is a critical need for further research to develop new treatment options for recurrent and metastatic PTC cases. Chalcones, belonging to the flavonoid family, are present in numerous plants. The addition of various functional groups to chalcone compounds can impart a wide spectrum of biological activities. Therefore, chalcones are important components for the development of new anticancer agents. Additionally, co-application of chalcones with other therapies is anticipated to enhance anticancer efficacy. HDACi (Histone Deacetylase Inhibitors) function by preventing the repression of tumor suppressor genes. Valproic Acid (VPA), prescribed for epilepsy and bipolar disorder, exhibits HDACi activity in various studies, impacting critical pathways including cell cycle regulation, apoptosis, and DNA repair.

Materials and Methods: In this study, SRB and ATP assays were used to investigate the cytotoxic effect of the combined treatment with the chalcone complex and VPA on the human papillary thyroid cancer cell line TPC-1. Triple fluorescent staining (Annexin V/Hoechst/Propidium Iodide) was used to determine the pathway of cell death. The transwell chamber and wound healing assays were utilized to assess the antimetastatic effect of the combined treatment.

Results: The combination of the chalcone complex and VPA increased the cytotoxic effect in TPC-1 cells. Staining analysis determined that the cell death pathway involves apoptosis. Importantly, this treatment was shown to suppress metastasis.

Conclusion: These results indicate that the combination of the chalcone complex and VPA could be effective in treating PTC, as it increases cytotoxicity, induces apoptosis, and suppresses metastasis.
This study is supported by the Bursa Uludağ University Research Fund under project number FYL-2024-1705.

Keywords: Thyroid Cancer, Metastasis, VPA, Chalcone

P065
Peritoneal B1 cells Increase Their Mitochondrial Membrane Potential and IL-10 Secretion Upon TLR Stimulation

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Background: B1 cells are tissue-resident and innate-like cells that are primarily located in the peritoneal and pleural cavities. In the literature, mitochondrial membrane potential (MMP) was significantly increased in wild-type (WT) mice B1a cells compared with the peritoneal B2 cells by using tetramethylrhodamine (TMRM). However, the effect of Toll-like receptor (TLR) ligands such as PAM3CSK4, and
Helicobacter felis (*H. felis*) on the MMP and IL-10 secretion levels of B1 cells is unknown. In our study, we aim to assess the effect of different TLR ligands (Lipopolysaccharides (LPS), PAM3CSK4 and *H.felis*) on the MMP and IL-10 secretion levels of B1 cells.

Materials and methods: Peritoneal cavity cells were isolated from IL-10-GFP reporter (VertX IL10^{egfp}) mice by peritoneal lavage. These cells were stimulated with TLR4-ligand LPS (5µg/ml), TLR2-ligand PAM3CSK4 (2,5 µg/ml) and *H. felis* (10µg/ml) for 72h. B1 cells were characterized by CD19 and CD23 surface marker stainings and Mitoview 633 was used to quantify MMP. Both MMP and IL-10 secretion were analyzed by flow cytometry. IL-10 secretion levels were further investigated by IL-10 ELISA.

Results: Our results showed that different TLR ligands, LPS, PAM3CSK4, and *H.felis,* have significantly increased the MMP and IL-10 secretion levels of B1 cells in the peritoneal cavity compared to the unstimulated cells at 72h. IL-10 ELISA results validated the findings obtained from flow cytometry.

Conclusion: These results suggest that *in vitro* stimulation of mouse peritoneal cells with TLR ligands (LPS, PAM3CSK4, and *H.felis*) enhances both MMP and IL-10 secretion levels. The elevated IL-10 levels imply that these cells are likely to acquire regulatory characteristics upon stimulation. Further studies are needed to explore the energy metabolism of B1 cells, and particularly B1-a cells.
This study was supported by the Health Institutes of Türkiye (TUSEB), Project Number: 32714.

Keywords: B1 cells, Peritoneal cavity, IL-10, Mitochondrial membrane potential

P066
Detection of Pestiviruses In Sheep by RT-PCR, Ag-ELISA and VNT Method In Algeria

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Background: Border disease virus (BDV) is a pestivirus responsible for significant economic losses in sheep industry. The present study was conducted between 2015 and 2016 to determine the flock seroprevalence of the disease in Algeria and to identify associated risk factors.

Materials and methods: 56 flocks from nine departments were visited and 689 blood samples were collected from adult sheep between 6 and 24 months of age (n=576) and from lambs younger than 6 months (n=113). All samples were tested by RT-PCR as well as by Ag-ELISA, to detect Persistently Infected (PI) animals. Serum samples from adults were tested by Ab-ELISA (Enzyme Linked Immuno-Sorbent Assay), to detect specific antibodies against pestivirus and 197 of them were further characterized by VNT (virus neutralization test) for the detection of BDV, BVDV-1 and BVDV-2 specific neutralizing antibodies.

Results: No PI animals were found among the 689 sheep tested. 144/197 sera were positive in VNT for BDV, and 2 sera were strongly positive BVDV-2. Fifty-five flocks (98 %) had at least one positive animal and the apparent within-flock seroprevalence was estimated to be 60.17% (95% C.I.: 52.96-66.96). The true prevalence based on sensitivity and specificity of the ELISA was 68.20% (95% C.I.: 60.2-76.3). Several risk factors were identified as linked to BDV such as climate, landscape, flock management and presence of other species in the farm.

Conclusion: These high seroprevalence rates suggest that BDV is widespread and is probably endemic all over the country. Further studies are needed to detect and isolate the virus circulating in the country and understand the distribution and impact of pestiviruses in the Algerian livestock.

Keywords: order Disease Virus; pestivirus; sheep; Algeria; Persistently Infected

P067
In Vitro Investigation of the Potential of Newly Synthesized Artemisia Alcohol Derivatives as Ezrin Inhibitors in the Treatment of Lung Cancer

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Background/Aim
Artemisia annua (A. annua) is a medicinal plant that has been used in traditional medicine for centuries for its various therapeutic properties, including anticancer activities. Artemisinin, the active component of the plant, has been shown to exhibit significant in vitro activity against several types of cancer, including small cell lung cancer.
With these promising results, further research is needed to evaluate its potential in in vivo models. According to current research; ezrin has been shown to function as a precursor protein of metastasis in lung adenocarcinoma cells, and its increased expression is associated with poor prognosis.
In this work, it was aimed to examine the ezrin binding potential of artemisia derivatives and to determine their effect in reducing Ezrin-CD44 binding.

2. Materials and Methods
2.1. Cytotoxicity Test
The cytotoxic effect of the organic compound Artemis Alcohol on lung cancer cell lines A549 and Calu-1 was determined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. A549 and Calu-1 cells were incubated at concentrations of 0-300 µM for 24 h. The concentration of essential oil that led to 50% inhibition of cell viability (IC50) was calculated using Prism V8 software.
2.2.Immunoprecipitation
Immunoprecipitation analysis was performed in a drug-treated environment with β-actin and CD44, which are associated with Ezrin, under these conditions, the binding potentials of Artemisia alcohols to Ezrin were examined.
2.3. Western Blot
The antibody interactions of β-actin and CD44 proteins, known to be associated with ezrin and Artemisia derivatives, were analyzed using Western blot analysis.
3. Results
Artemisia organic compounds showed dose-dependent cytotoxic effects on lung cancer cells. IC50 values were 218 µg/ml for A549, 36 µg/ml and 74 µg/ml for Calu-1.
4. Discussion
Artemisinin complexes obtained from Artemisia annua showed anticancer activity to lung cancer cell line. Dose-dependent cytotoxicity in A549 and Calu-1 cells highlights their therapeutic potential. Further in vivo studies are needed to confirm these results and explore clinical applications.

Keywords: Lung cancer, Artemisia, Anticancer, Essential oil, Ezrin

P068
Prevalence and Molecular Characterization of Cryptosporidium in Rabbits from the Local Algerian Population

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Background/aim: In rabbits, parasitic infection is one of the main constraints of the development of rabbit production, particularly, endoparasites. Cryptosporidiosis is one of a cosmopolitan parasitosis caused by numerous species belonging to the Cryptosporidium genus. Regardless of the severity of their symptoms, they have a fundamental economic impact on rabbit farming, the rabbit industry and public health. Since there is no infection data in rabbits, the aim of this study is to estimate the prevalence, risk factors and molecular characterization of Cryptosporidium spp. in rabbits from the local population.

Materials and Methods: 5 districts were studied. Faecal samples were collected from (n=638) and examined by modified Ziehl-Neelsen technique for the presence of Cryptosporidium oocysts. The positive samples were genotyped and subtyped to determine the circulating Cryptosporidium species.

Results: The overall prevalence of Cryptosporidium spp. was 24% (153/638). Higher prevalence was recorded in Blida district (57%), in females (63%), kits after weaning (30%) and in rabbits keep in poor hygienic condition (40%). Also, using food made up of household leftovers (peelings, bread, fruit and vegetables) or cohabitation has increased the prevalence of Cryptosporidium spp. 3 species were identified: Cryptosporidium cuniculus (54.28%), Cryptosporidium parvum (22.85%) and Cryptosporidium murus (2.85%). However, sequencing of the Cryptosporidium parvum genotype allows identifying the IIcA5G3 subtype in rabbits during the fattening period.

Conclusion: In conclusion, the majority of farms have rabbits contaminated by Cryptospridium. Particular attention should therefore be paid to preventing infection of humans and avoiding pollution of the environment.

Key words: Cryptosporidium, genotyping, rabbit, zoonosis.

P070
The Role of NF-κB in the Proliferation and Differentiation of HaCaT Cells Containing Cx26 KID Syndrome Mutations

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Background/Aim: Keratitis-ichthyosis-deafness (KID) syndrome is a rare genetic disease characterized by deafness, visual impairments, and palmoplantar keratoderma. The disease arises from Connexin26 (Cx26) mutations that lead to the formation of hyperactive hemichannels causing uncontrolled molecule transfer across the membrane. Although the uncontrolled molecule transfer affects the proliferation and differentiation of keratinocytes, the exact molecular and cellular mechanisms underlying the epidermal alterations due to Cx26 mutations are not yet known. Previous studies showed enriched NF-κB pathway members in keratinocyte cell line HaCaT containing Cx26-D50Y KID syndrome mutation. Therefore, we investigated the role of the NF-κB signaling pathway on the proliferation and differentiation mechanisms of the HaCaT cells that constitutively express the Cx26-G45E and Cx26-D50Y mutations.

Materials and Methods: Nuclear localizations of NF-κB transcription factors were compared in HaCaT cells expressing Cx26-WT, D50Y, or G45E by immunostaining to compare NF-κB activity. HaCaT cells were treated with sodium salicylate, an NF-κB inhibitor, to examine the effects of NF-κB pathway on the proliferation and differentiation of the cells by using MTT analysis, BrdU assay, cell cycle and apoptosis analysis, Q-RT-PCR, Western blotting, and immunostaining.

Results: G45E and D50Y cells showed the highest nuclear RelA and c-Rel signals, respectively. In addition, NaSal treatment affected early and late apoptosis rates differently in D50Y cells. Moreover, G45E and D50Y cells had opposite trends when early and late apoptosis rates were compared. Furthermore, NF-κB inhibition decreased proliferation rate of G45E cells, unlike WT cells. Lastly, cytokeratin10 protein levels showed differences in G45E cells after NaSal treatment.

Conclusion: NF-κB may be activated through different NF-κB members in G45E and D50Y. In addition, NF-κB pathway may influence apoptosis in G45E and D50Y cells through different mechanisms. The NF-κB pathway may also regulate proliferation and differentiation mechanisms of G45E cells, implicating the differential effects of mutations in HaCaT cells.

Keywords: connexin26, Keratitis-ichthyosis-deafness KID syndrome, epidermal differentiation, proliferation, NF-kB signaling pathway

P071
Development and Characterization of Solanum linnaeanum Hairy Root Lines

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Background/Aim:*Solanum linnaeanum* is a wild *Solanum* species and used traditionally for therapeutic purpose due to its secondary metabolite content including phenolics and glycoalkaloids which have various biological activities. Hairy root culture (HRC) is a technique used for secondary metabolite production. HRCs are generated by transformation with *Agrobacterium rhizogenes*, which possesses a root-inducing plasmid (Ri plasmid) and infects the plant, thus, inducing hairy root growth. The study aimed to generate HRCs of *S.linnaeanum* and *S. melongena* to characterize the secondary metabolites produced by *S. linnaeanum* and to compare it with HRCs of *S. melongena*.

Materials&Methods and Results: In this study, one agropine strain (ATCC 43057) of *A. rhizogenes* was used to generate HRCs. Then, T-DNA integration was verified by PCR with two different *rol* gene primers and verified lines were grown in liquid culture. Subsequently, biochemical analysis of HRCs and non-transformed tissues were carried out. The average total phenolic contents were found as 5.75 and 3.83 mg gallic acid equivalent (GAE)/g for *S. linnaeanum* and *S. melongena* HRCs, respectively. The average flavonoid contents were found as 14.85 and 9.32 mg (+)-catechin equivalent (CE)/g for *S. linnaeanum* and *S. melongena* HRCs, respectively. The average total antioxidant capacities of *S. linnaeanum* and *S. melongena* HRCs were found as 11.45 and 9.76 mg Trolox equivalent (TE)/g, respectively. Expression analysis of *myb1*,
HQT, and *Game9* genes in leaves and HRCs of *S. melongena* and *S. linnaeanum* was performed with RT-PCR and the mRNA expression level of *myb1* and *Game9* genes was significantly higher in HRCs of *S. linnaeanum* compared to *S. melongena* HRCs. Similarly, total phenolic and flavonoid content and total antioxidant capacity were significantly higher in HRCs of *S. linnaeanum*.

Conclusion: In conclusion, our results indicates that HRCs of *S. linnaeanum* can be a promising source for secondary metabolite production.

Keywords: Solanum melongena, Solanum linnaeanum, Hairy root culture, Plant secondary metabolites, Phytochemical analysis

P072
The Use of CRISPR/Cas9 Technology to Reduce Polyphenol Oxidase (PPO) Enzyme Activity in Eggplant

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Background/Aim: While Turkey ranks among the top five eggplant producers worldwide, its exports are limited due to issues like enzymatic browning caused by bruising or processing of the fruits. Enzymatic browning negatively impacts the fruit's quality by altering its smell, appearance, and nutritional composition. The enzymes responsible for browning are polyphenol oxidases (PPO), released from damaged cell's plastids, which catalyze phenolic oxidation. The objective of this study is to introduce mutations into the PPO genes, specifically expressed in eggplant fruit, aiming to reduce expression of PPO genes and enzyme production.

Materials and Methods: In this study, two different CRISPR/Cas9 cassettes will be prepared to target the Polyphenol Oxidase genes to induce a premature stop codon or similar mutation in the genes. One of the constructs will contain two different guide RNAs to target three of 13 PPO genes in eggplant and the other construct will contain three different guide RNAs to target 10 of 13 PPO genes. The constructed CRISPR/Cas9 cassettes will be transferred to eggplant hypocotyls by *Agrobacterium tumefaciens* mediated gene transfer. Furthermore, the PPO genes of edited plants will be sequenced to determine their specific mutations. The plants will be analyzed for PPO gene expression, enzyme activity, chlorogenic acid content and total antioxidant capacity.

Results:For hypocotlys which transformed with 2 gRNA carrying casette,13 of 30 regenerated plants were found to be positive for T-DNA integration. Sequencing studies are ongoing to detect mutations in each targeted PPO genes. Studies on cassette preparation for the system carrying 3 guide RNAs are continuing.

Conclusion: When this study is completed, a long-term solution to the browning problem will be offered by regulating PPO genes in Turkish type eggplants. Physical properties and nutritional value preserved, shelf-life extended eggplants will be easily consumed out of season and outside the local growing region.

Keywords: Eggplant, Polyphenol Oxidase PPO, Genome Editing, CRISPRCas9, Fruit Browning

P073
SEMA5A Downregulation Might be a Prognostic Factor in Head and Neck Cancer Aggressiveness

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Background/Aim: Oral squamous cell carcinoma is the most common type of head and neck cancer(HNSCC), with tongue cancer being the most aggressive subtype. Despite a decreasing overall incidence of head and neck cancers due to reduced cigarette and alcohol consumption, there is a specific and concerning rise in the incidence of tongue cancer. Semaphorin-5A(SEMA5A), initially recognized as an axon guidance molecule, has emerged as a prognostic and predictive biomarker in various cancers. However, SEMA5A exhibits multifaceted roles in cancer biology, acting as a tumor suppressor in glioma while promoting tumor progression in gastric cancer. Additionally, SEMA5A regulates the metastatic potential and plays a crucial role in organ-specific homing of pancreatic cancer cells. Given that SEMA5A has a significant involvement in metastasis, this study aims to investigate its implications in HNSCC.

Materials and Methods: The correlation between the SEMA5A expressions and nodal metastasis/tumor grade/HPV status is evaluated in TCGA and CPTAC head and neck cancer cohorts by using UALCAN(http://ualcan.path.uab.edu) database. The SEMA5A expression in tongue cancer cell lines, HSC-3, UM-SCC6, UM-SCC47, and SCC25 was compared to a healthy gingival cell line(HGF-1) by qPCR. Orospheres are formed and evaluated for their cancer stem cell-related gene and SEMA5A expression.

Results: SEMA5A expression is significantly reduced in grade 3 and N3 nodal metastatic HNSCC, correlating with decreased overall survival. HPV-negative HNSCC patients show lower SEMA5A expression compared to HPV-positive counterparts. SEMA5A is upregulated in primary tongue cancer cell lines(UM-SCC6, UM-SCC47, SCC25) compared to HGF-1 cells, while downregulated in metastatic cell line HSC-3. Furthermore, SEMA5A expression is diminished in cancer stem cell-enriched orospheres, suggesting a negative correlation between SEMA5A expression and tongue cancer aggressiveness.

Conclusion: SEMA5A downregulation correlates with stemness, metastasis, and patient outcomes, highlighting its potential as a prognostic biomarker and therapeutic target in tongue cancer.

Keywords: SEMA5A, Metastasis, Head and Neck Squamous Cell Carcinoma, Oral Squamous Cell Carcinoma

P074
Comparison of Brain Regeneration and Brain Cancer at the Transcriptomic Level on Zebrafish Model

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Background/Aim: Glioblastoma (GBM) is defined as the tumor in the brain which has formed initially by glial cells. It has a high mortality rate, and it is virtually impossible to cure completely. New approaches to the cancer research should be taken into account to understand the disease and for the potential therapies. In this regard, we take cancer as "a wound that does not heal". Understanding the similarities and differences between a healing wound and cancer can provide potential targets for therapy. According to our previous research, there are transcriptomic similarities between human GBM cells and regenerating zebrafish brain cells. We wanted to enhance this research by comparing cancer and regeneration in the same organism with a deeper understanding.

Materials and Methods: To generate brain tumors, we manipulate zebrafish embryos at single-cell stage with an oncogene plasmid (UAS:HRASV12:GFP) which creates scattered tumors throughout the brain. To comparatively analyze, we use stab wound injury technique to generate regenerative brain model. Our aim is to perform RNA sequencing with both brains and find genes that act in opposite directions at certain stages of cancer and regeneration. Our next goal is to alter the expression of candidate genes on human GBM cell lines via CRISPR-Cas9 and observe them with in vivo xenograft models.

Results: Based on our studies, we have successfully generated both models. Morphologic and molecular validations support our further studies. RNA sequencing data will give us more insights about the mechanisms of both models.

Conclusion: We hypothesize that leveraging this shared transcriptomic landscape in zebrafish can facilitate the reprogramming of brain cancer cells, redirecting their hyperactive healing tendencies towards a controlled regenerative state. These genes can be critical in distinguishing between regeneration and cancer. Our research has an important value due to its different approach methods to cancer and its potential therapeutic side.

Keywords: glioblastoma, cancer, regeneration, zebrafish, brain

P075
Comparative Analysis of Long-Read SV Calling Tools: Evaluating the Subtraction Method for Detecting Somatic Structural Variants in Cancer Genomes

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Background/Aim: Cancer genomes have a complicated landscape of mutations, including large-scale rearrangements known as structural variants (SVs). These SVs can disrupt genes or regulatory elements, playing a critical role in cancer development and progression. Despite their importance, accurate identification of somatic structural variants (SVs) remains a significant bottleneck in cancer genomics. Long-read sequencing technologies hold great promise in SV discovery, and there is an increasing number of efforts to develop new tools to detect them.

Materials and Methods: In this study, we employ eight widely used SV callers on paired tumor and matched normal samples from both the NCI-H2009 lung cancer cell line and the COLO829 melanoma cell line, the latter of which has a well-established somatic SV truth set. Following separate variation detection in both tumor and normal DNA, the VCF merging procedure and a subtraction method are used to identify candidate somatic SVs. Our analysis adopts a comprehensive approach, evaluating the performance of each SV caller across a spectrum of variant types and numbers in finding cancer-related somatic SVs. Additionally, we explore different combinations of these tools to enhance the accuracy of true somatic SV detection.
Results: Given the rapid development of long-read SV analysis tools recently, a thorough comparison of these tools becomes as important as their development. This study, by comparing eight different tools and their combinations, not only reveals the benefits and limitations of various techniques but also establishes a framework for developing more robust SV calling pipelines for cancer research.

Conclusion: Our findings highlight the strengths and weaknesses of current SV calling tools and suggest that combining multiple tools and testing different combinations can significantly enhance the validation of somatic alterations. This approach will improve the detection accuracy of somatic SVs in cancer genomes, providing a more reliable foundation for subsequent research and clinical applications.

Keywords: Cancer Genomics, Long-Read Sequencing, Somatic Structural Variants, SV Calling Tools

P076
Two-way Regulatory Role of NKX3.1 on Epithelial-Mesenchymal Transition Factors in the Inflammatory Microenvironment of Prostate Cancer

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Introduction: Prostatic inflammation leads to many tumorigenic alterations contributing to cancer initiation, progression, and metastasis. Loss of NKX3.1 due to its inflammation-mediated proteasomal degradation results in prostate carcinogenesis (PCa). NKX3.1 is a tumor suppressor and has various regulatory roles in antioxidant response, cell growth, epithelial-mesenchymal transition (EMT), and stemness. Although lack of NKX3.1 expression is strongly correlated to prostate cancer development, metastatic cells exhibiting recovered NKX3.1 expression raised the question that NKX3.1 may distinctly regulate EMT mechanisms in the normal and inflammatory tumor microenvironment. In this study, we aimed to research the effect of NKX3.1 expression on inflammation-induced EMT factors in prostate cancer cells.

Material and Methods: LNCaP (androgen-responsive), PC3, and DU145 (castration-resistant) prostate cancer cells were treated with inflammatory conditioned media (CM) w/w.o. NKX3.1 overexpression. Alterations in EMT factors were determined by western blot and qRTPCR array system and colocalization of EMT factors was analyzed by immunofluorescence. Cell proliferation and migration were ultimately assayed by WST-1 and the transwell migration system.

Results: LNCaP, PC3, and DU145 cells treated with CM for 8 days showed a mesenchymal-like morphology. Further, N-cadherin, fibronectin, TWIST1, and SNAIL1, which majorly regulate EMT and cellular migration were detected to be upregulated due to NKX3.1 overexpression under normal cellular state, however, suppressed under inflammatory conditions. qRTPCR array results revealed that primary EMT factors such as SNAIL1, AHNAK, MSN, SPP1, IGFBP4, MMP2, MMP9, SOX10, GSC4, MST1R, BMP1, SOX10, and SPARC were regulated by NKX3.1 in opposite ways in the absence or presence of inflammatory microenvironment. Cellular migration was detected to be suppressed by NKX3.1 under both conditions.

Conclusions: In conclusion, NKX3.1 regulates many EMT factors in the inflammatory microenvironment of prostate cancer differently from its cellular functions. Although our results support that NKX3.1 suppresses prostate cancer cell migration, distinct regulation of EMT factors suggested a context-dependent role of NKX3.1 under inflammation.

Keywords: EMT, PCa, NKX31, TWIST1, inflammation

P077
Biocompatible and Safe Decellularized Spinach With Wound Healing Activity

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Background/Aim: Eliminating cellular components from a tissue or organ while preserving extracellular matrix in a biocompatible and safe environment for cell growth, is the primary goal of decellularization.
Challenges and drawbacks associated with traditional decellularization techniques have motivated the search for more efficient methods. Optimization of decellularization is indeed crucial. We aimed in this work to create acellular scaffold possessing a wound healing activity with minimal concentration of available detergents, mainly, Tween-20.

Methods: Spinach leaves were decellularized at 21°C and 37°C with different concentrations of Tween-20 of 0.5%, 1% and 5% associated with 1%SDS and 1%Sodium hypochlorite. Decellularized lyophilized constructs were sterilized with PBS and UV for in vitro experiments.
DNA and protein quantification were determined. MTT assay was used to assess the biocompatibility of decellularized constructs on A549, NIH/3T3 and BEAS-2B cell lines Scratch assay was performed to evaluate the wound healing activity at different time points.

Results: MTT analysis of samples treated with 1% Tween at 21°C and 37°C for 24 hours and 48 hours showed improved cell viability in NIH/3T3 and Beas2B cells, especially in the group exposed to 1% Tween at 21°C compared to the control group. The results were statistically significant. Migration test of decellularized scaffold with 1% Tween at 21°C and 37°C was performed in NIH/3T3 cell line. Cells exposed to 1% Tween at 21°C showed improved wound healing activity with NIH/3T3.

Conclusion: This work developed an effective and cost-effective decellularization protocol using minimal concentrations in a short time frame. The protocol did not show cytotoxic effects on cell lines as a result of MTT testing with NIH/3T3. Therefore, if decellularized tissues support cell migration, they can inevitably be considered as an excellent option for wound healing patches.

Keywords: Decellularization, Decellularized scaffolds, Spinach, Plant tissues

P078
Streptozotocin-Induced Type 1 Diabetes in Mice: Unveiling Muscle Atrophy Gene Activation

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Background/Aim
Type 1 diabetes (T1D) is an autoimmune condition characterized by the destruction of insulin-producing βcells. This study aims to induce T1D in mice using streptozotocin (STZ) and to analyze the expression of atrophy-related genes in muscle tissue. Understanding the molecular mechanisms underlying muscle atrophy in diabetic conditions could provide insights into potential therapeutic targets.

Materials and Methods: Male C57BL/6 mice were injected with 50mg/kg/day STZ to induce T1D. Blood glucose levels were monitored to confirm diabetes onset. Post-induction, grip strength and hanging measurements were performed to assess baseline muscle function. Post-induction, tissues were harvested and weighed. RNA was extracted and reverse transcribed to cDNA. Quantitative PCR was performed to measure the expression levels of atrophy-related genes, including MuRF1, Atrogin-1. Data were analyzed using statistical software.

Results: STZ-treated mice showed significantly elevated blood glucose levels, confirming the successful induction of T1D. Hanging and grip strength-tests indicated a decrease in muscle function. Analysis of muscle tissue post-induction revealed a marked increase in the expression of MuRF1 and Atrogin-1 compared to control mice. These findings indicate a clear link between T1D and the activation of atrophy pathways in muscle tissue.

Conclusion: The induction of T1D in mice using STZ results in the upregulation of atrophy-related genes in muscle tissue. These results highlight the potential impact of diabetes on muscle health and underscore the importance of targeting atrophy pathways in the development of treatments for diabetes-related complications.

Keywords: Type 1 Diabetes, Streptozotocin, Muscle Atrophy, Gene Expression, Muscle Function

P079
A Novel Curcumin-Derivative Activity Against Sodium Arsenate Toxicity In Vitro in CCD-1064Sk Human Dermal Fibroblast Cell Line

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Background/Aim: In this study, the protective effects of the newly synthesized curcumin derivative compound named curcumin ester against arsenate toxicity induced *in vitro* in fibroblast cells investigated. Healthy human skin fibroblasts (CCD-1064Sk) were chosen, as they play a significant role in maintaining the structural integrity of most tissues in the human body, to examine the cytotoxicity of sodium arsenate. This chemical may trigger oxidative stress, the destruction of mitochondria, and an induction of cell death pathways.

Materials and Methods In the current research, in the presence of sodium arsenate in the concentration range of 0-500 µM, a 0-200 µg/mL curcumin ester was examined. To understand the cell death mechanism, the acridine orange/ethidium bromide and DAPI (*4',6-diamidine-2'-phenylindole dihydrochloride*) staining techniques were applied using a fluorescence microscopy. The gene expression of the apoptosis-related gene caspase-9 was analyzed using real-time polymerase chain reaction.

Results: According to results, it was determined that sodium arsenate at the concentration of 50 µM killed 48.5 percent of human fibroblast cells in 24-hour period. Cell viability was determined to be 59% in cells exposed to 200 µg/mL concentration of curcumin ester for 48 hours. Examination of morphological changes in cellular and nuclear structures showed a notable inhibition of the cellular network loss associated with a novel curcumin derivative treatment and that derived substance protects cells against toxicity caused by sodium arsenate. The real-time polymerase chain reaction showed that the caspase-9 gene was overexpressed in groups that were exposed to arsenate and treated with different concentrations of curcumin ester. The untreated, arsenate-exposed group showed downregulation of the caspase-9 gene.

Conclusion: Our research has demonstrated that sodium arsenate exposure leads to significant alterations in fibroblast cell morphology and viability. In conclusion, a curcumin-derivative compound, a newly synthesized unique molecule, may have a protective effect against arsenate toxicity.

Keywords: antioxidant, arsenate, curcumin, toxicity, viability

P081
Macular Corneal Dystrophy Disease Model in Zebrafish Generated via Carbohydrate Sulfotransferase 6 (chst6) Mutagenesis

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Background/Aim: Macular corneal dystrophy (MCD) is a rare congenital disease caused by mutations in the
carbohydrate sulfotransferase 6 (chst6) gene. Patients suffer from opaque aggregates in the cornea, causing vision loss in the 4th decade of life. Corneal transplantation is the only available treatment, not available to every patient. Keratocytes in the cornea express the *chst6* gene, which encodes enzyme that is essential for sulfation of the keratan sulfate proteoglycans (KSPG). The loss of KS sulfation leads to defects in collagen fibril organization and aggregate formation in the corneal extracellular matrix. Lack of preclinical disease models is a major limitation for the development of accessible treatment strategies. Zebrafish cornea structure is highly like humans, containing high levels of KSPGs in the stroma. Here, we aimed to develop an MDC model in zebrafish.

Materials and Methods: *chst6* gene was edited via CRISPR/Cas9. Mutations were identified with Sanger sequencing, and loss-of enzyme function was tested with ELISA and immunofluorescence staining. Eyes of the mutant fish analysed microscopically and histopathological. Alcian blue staining was used to assess aggregate formation. Skeletal structure was analysed with alcian blue and alizarin red staining of the larvae and adults respectively.

Results: Several *chst6* alleles were obtained and loss of KSPG sulfation in the mutants was demonstrated. Age-dependent opaque deposits that were positive for alcian blue formed on the cornea of mutant zebrafish. It was shown that there were alterations in epithelium thickness and corneal structural degeneration. A small fraction of mutant fish showed defects in jaw cartilage, adult jaw and vertebra morphology.

Conclusion: The first *in vivo* model of MCD is generated in zebrafish, and gene-disease relationship is proven in vivo. This model exhibits all corneal symptoms of the disease and can be used as a preclinical model. Moreover, additional roles of *chst6* gene in cartilage and skeleton development is uncovered.

Keywords: Zebrafish, Macular Corneal Dystrophy, Carbohydrate Sulfotransferase 6, Keratan sulfate proteoglycan, Preclinical Model

P082
Development and Implementation of Recombinant Anti-Freeze Protein for Anti-Icing Applications

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Background/Aim: Ice formation on a solid surface poses a significant challenge in various industrial settings, resulting in higher energy consumption, decreased performance, and destructive consequences [1]. The development of functional surfaces resistant to icing, which inhibit ice formation, is essential for minimizing operational expenses and prolonging the surface's lifespan [2]. Employing cryoprotectants to prepare anti-icing surfaces represents a highly efficient approach that finds applications across diverse fields. Natural cryoprotectants such as antifreeze proteins (AFPs) are utilized to produce anti-icing surfaces capable of lowering the freezing point and inhibiting ice crystal formation through the mechanisms of thermal hysteresis (TH) and Ice Recrystallization Inhibition (IRI) [3].

Materials and Methods: In the current study, recombinant protein technology was utilized to process molecular cloning, expression, and synthesis of an AFP from *E. coli*. The purification of the recombinant AFP was done by his-tag affinity chromatography. The characterization tests of expressed AFPs were conducted by 3D structure modeling, SDS-PAGE, and CD Spectroscopy analysis. Thereafter, the AFPs were coated on the aluminum surfaces by oxygen plasma. The anti-icing activity of the recombinant AFP coated on aluminum surfaces was validated in the developed experimental setup. Scanning Electron Microscopy (SEM) analysis and Contact Angle (CA) measurements were employed for the characterization of the recombinant AFP-coated aluminum surfaces.

Results: SDS-PAGE analysis and CD-spectroscopy results revealed that the size of the his-tag purified AFP was around 63 kDa and had an α-helical structure, respectively. The anti-icing tests proved that the recombinant AFP-coated aluminum surfaces altered and delayed the ice formation on metallic surfaces whereas the aluminum bare surfaces had significant ice formation in the first 10 seconds of the test.
Conclusion: The results of this investigation offer new perspectives on the biotechnological utilization of AFPs in the energy applications refrigeration and air conditioning, promoting energy efficiency and savings and contributing to tackle with global warming.

Keywords: Antifreeze Proteins, Ice Binding Proteins, Antifreeze Activity, Antifreeze coating

P083
Development and characterization of melittin-modified niosomes

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Niosomes are vesicular carriers formed through the self-assembly of surfactants and cholesterol, representing promising drug delivery systems. Unlike liposomes, they can be produced on a large scale at a lower cost and offer superior chemical and physical stability, making them more robust for various applications. In our previous study, we optimized niosomal formulations and identified the best noisome formulation as a 1:1 molar ratio of Span 60 and cholesterol at a final concentration of 0.1 mM for cellular applications. In this study, we are functionalizing the niosomes with a peptide called melittin, the primary component of bee venom known for inducing cell cycle arrest and apoptosis in cancer cells, thus holding promise for cancer treatment.

The noisome formulations with melittin are characterized using dynamic light scattering (DLS) to determine size distribution and zeta-potential, Fourier-transform infrared spectroscopy (FTIR) for compositional analysis, and scanning electron microscopy (SEM) for morphological evaluation. Furthermore, we assess the *in vitro* efficacy of the functionalized niosomes is assessed through a cytotoxicity assay. This assay compares the effects of free melittin, scrambled melittin (as a control), and melittin-loaded niosomes on both healthy endothelial cells (HUVEC) and cancerous cells (U87). The investigation encompasses a range of concentrations (1.25 to 10 μg/ml) and time points (6, 24, and 48 hours) to comprehensively evaluate the therapeutic potential of this novel drug delivery system.

Keywords: niosome, melittin, surface modification

P084
Pyrrlodine Dithiocarbamate Protects against Cisplatin-Induced Liver Damage by Regulating NF-κB Activation

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Background/Aim: Cisplatin is a synthetic, platinum-based chemical agent used in cancer chemotherapy. Pyrrolidine dithiocarbamate (PDTC), known for its metal-chelating properties, is a synthetic chemical compound belonging to the dithiocarbamate family, which exhibits dose-dependent antiviral, anticancer, antibacterial, antioxidant, and anti-inflammatory effects. In our study, we aimed to elucidate the effects of PDTC on cisplatin-induced liver damage in the model organism, the rat.

Materials and Method: We formed four experimental groups of 2-3 month-old 32 male *Wistar albino* rats. Control group animals were injected with physiological saline solution, while the second group was intraperitoneally injected with 100 mg/kg PDTC for 7 days once a day. The third and fourth experimental groups were injected with the indicated PDTC dose, along with an injection of 7 mg/kg cisplatin on the third day. After the 7 days of the experimental period, liver samples obtained from the animals were analyzed using spectrophotometry to measure aspartate aminotransferase (AST) and myeloperoxidase (MPO) activities, as well as malondialdehyde (MDA) and glutathione (GSH) levels. Additionally, phosphorylation of the NF-κB transcription factor was determined by western blotting method.

Results: This study showed that cisplatin administration results in liver damage by increasing the levels of tissue markers such as AST, MPO, and MDA, while causing a decrease in GSH. Additionally, the increase in NF-κB phosphorylation can be observed as an indicator of inflammatory activity in the cisplatin group. When compared to the cisplatin group, pre-treatment of PDTC resulted in decreased AST and MPO activities, as well as reduced MDA levels, while enhancing the levels of GSH in liver tissues. PDTC treatment also demonstrated its anti-inflammatory effect by reducing the levels of phosphorylated NF-κB.

Conclusion: In conclusion, this study shows that PDTC acts through the regulation of NF-κB activation in protecting against cisplatin-induced liver damage. In addition, PDTC can be considered a chemical agent that may prevent chemotherapy-induced liver injury.

Keywords: Cisplatin, pyrrolidine dithiocarbamate, liver damage, rats, NF-kappa B activation

P085
Development of Lipid Nanoparticles for Downregulation of SUV39H1 in Human Lung Cancer Cells

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Background and aim: Cancer still remains a disease with no definitive cure. RNA interference enables the investigation of new treatment methods by silencing genes that trigger cancer formation. A potential target gene for therapeutic silencing is the histone-lysine N-methyltransferase, SUV39H1 which is overexpressed in cancer. The efficacy of nucleic acid therapeutics is limited by the poor stability of nucleic acids in physiological fluids and limited cellular uptake. The aim of this study was to design a SUV39H1 siRNA and lipid nanoparticle (LNP) formulations to enable effective delivery.
Materials and methods: SUV39H1 transcript variant 1 sequence from NCBI was used to design the siRNAs employing iScore Designer web tool. Cationic LNP's were prepared by lyophilization-rehydration method. siRNA was complexed with LNPs electrostatically. Complex formation and stability against nuclease degradation was confirmed by gel retardation assay. LNPs and LNP:siRNA complexes were characterized in terms of size and zeta potential. Cytotoxicity of the formulations was evaluated on H1299, HCC827 and Calu1 cell lines. Cellular internalization was observed in Calu1 lung cancer cells.
Results: Two siRNA candidates were selected based on the iScore Designer scores. LNPs with particle size of 62.4 – 87.4 nm and zeta potential of +30.4 to +31.7 mV. It was observed that above N/P ratio of 10 the LNP formulations were able to form complexes with the siRNA. The complexes had sizes below 100 nm. Increasing N/P ratio increased the anionic stress resistance of the complexes. No degradation of the siRNA was seen in the presence of RNaseA. No toxicity was observed on the tested cells. Cellular uptake was optimal at the N/P 15.
Conclusion: The developed LNP:siRNA complexes show good in vitro stability, biocompatibility and cellular uptake. Further investigations of SUV39H1 silencing on lung cancer cells are warranted.
This study was supported by the Scientific Research Fund of Ege University (project no: 32149)

Keywords: siRNA, cancer, nucleic acid delivery

P086
In Silico Screening of New Delhi metallo- β – lactamase-1(NDM-1) Inhibitors with Drug Reporposing Strategy

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Atatürk Üniversitesi

Background/Aim: Excessive and improper use of antibiotics in both agricultural and clinical areas has resulted in a rise in bacterial strains that are resistant to several drugs. Among these strains, gram-negative bacteria that produce β-lactamase have become the most common. Given the significant role of metallo-β-lactamases in biomedicine, it has become imperative to identify inhibitors for these enzymes. NDM1 has exhibited the most rapid and extensive global dissemination among all metallo-β-lactamases, leading to the rapid emergence of bacterial infections containing plasmid-encoded NDM1 worldwide. Therefore, the goal of this study is to
discover novel and potent inhibitors of NDM1 by employing virtual screening techniques on existing drugs.

Materials and Method: For screening, the 4EXS crystal structure was selected based on information from the literature. All compounds (10767) in the Drugbank database were downloaded and prepared using the LigPrep module. The docking analyses were conducted with Glide's HTVS/SP/XP docking modules to identify compounds with the highest protein binding, and their effects on the *E. coli* strain containing the NDM-1 plasmid were assessed and the minimum inhibitory concentrations (MIC's) determined. Also, the synergistic effects of the drugs were examined with the checkerboard method.

Results: In the virtual screening, it was determined that NADH, Pemetrexed, and Zoledronic acid were hit drugs. The effects of the drugs were examined on the resistant *E.coli*
strain, and Zoledronic acid was found to be the most effective. MIC values were determined as Ampicillin512, Meropenem16, Imipenem256, Cefotaxime64, NADH>1024, Zoledronic Acid128, and Pemetrexed>1024. Zoledronic acid was found to have additive effects with Ampicillin, synergistic effects with Meropenem, indifference effects with Imipenem, and additive effects with Cefotaxime with the checkerboard method.

Conclusion: This study has shown that Zoledronic acid and Meropenem can be used in combination against
E.coli strains containing NDM-1 plasmid and that they may be potential therapeutic agents.

Atatürk University BAP unit (ProjectID:FBA-2020-8419) financed this research.

Keywords: NDM-1, Virtual Screening, Drug Reporposing, Ecoli, In Vitro

P087
In Vitro Study of NOD2 in Viral Immunity in Human Eosinophil-like Cells, Which Has Been Implicated in Bacterial Infections and Immune Diseases

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Background/Aim: Eosinophils are granulocytes of innate immune system. Although they are associated with parasitic infections, asthma and allergic diseases, they are also known to be involved in bacterial and fungal infections. Moreent studies have suggested that eosinophils may also play a role in viral infections. Eosinophils, which express pattern recognition receptors (PRRs), sense pathogen-associated molecular patterns (PAMPs) and mount an immune response by secreting granular proteins and cytokines. One of these PRRs is NOD2 located in the cytosol. NOD2 is known to involve in the development of anti-bacterial immune responses in macrophages and dendritic cells, however the role of NOD2 in eosinophils during viral infections is still unclear. Another cytosolic receptor, RIG-I, plays vital roles in the generation of anti-viral responses, however the function of these receptors in eosinophils remains elusive. Such cytosolic receptors are usually activated as multimeric protein complexes and influence the magnitude and duration of immune responses. In this context, there might be a relationship between NOD2 and RIG-I. The aim of this study is to understand the role of NOD2, RIG-I and downstream effectors in human eosinophil cells during viral infections.

Materials and Methods: To elucidate the viral responses of eosinophils, NOD2, RIG-I and downstream molecules' expressions were analyzed after stimulation with TLR7/8 and TLR8 ligands (R848 and ssRNA40) in EoL-1 human eosinophilic cell line.

Results: Differential expressions of proteins and downstream molecules were determined in response to R848 and ssRNA40 stimulation inEoL-1 cells.

Conclusion: NOD2 in collaboration with RIG-I, may also have roles during viral infections in human eosinophil cells.

This project was supported by ITU-BAP-44121.

Keywords: NOD2, antiviral immunity, viral infection, eosinophils

P088
Investigation of the Expression Levels of HTR1B and Its Ortholog ser-4 Genes in Two Different Models under Hypoxic Conditions

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Abstract
The *HTR1B* gene encodes a serotonin receptor and its *Caenorhabditis elegans* ortholog gene, *ser-4*, codes for a protein that activates G protein-coupled serotonin receptors. This study aims to observe the changes in serotonin receptors under hypoxic conditions and compare their regulation across different organisms. The responses of the *HTR1B* gene in HUVEC cells (Human Vein Endothelial Cells) treated with cobalt chloride ($CoCl_2$) and *ser-4* gene in *C. elegans* exposed to sodium sulfite (Na_2SO_4)-induced chemical hypoxia were evaluated.
HUVEC cells were cultured in DMEM (Dulbecco's Modified Eagle's Medium) containing 10% FCS (Fetal Calf Serum) and hypoxia was induced with 150 µM $CoCl_2$. After total RNA isolation, cDNA was synthesized and firstly, HIF-1αmRNA level was evaluated by Real-Time PCR method to confirm hypoxic condition. HTR1B mRNA level was compared under hypoxic conditions compared to normal conditions.
NGM (Nematode Growth Medium) containing supplements for C. elegans was prepared and OP50 strain was cultivated. Chemical hypoxia was created with Na_2SO_4 (Sodium Sulfite). Total RNA was isolated and cDNA synthesed. ser-4 mRNA expression was evaluated with the Real-Time PCR method.
It was observed that HIF1-α expression increased in the HUVEC cell line at 24, 48 and 72 hours. It was determined that HTR1B mRNA level increased under hypoxic conditions at 48 and 72 hours. In *C.elegans*, it was determined that hypoxia with Na_2SO_4 occurred only at 1 hour and ser-4 mRNA level also increased.
As a result, it was observed that hypoxic conditions occurred in all time intervals in the HUVEC cell line and the HTR1B gene responded to hypoxia. In *C. elegans*, hypoxia occurred only at 1 hour, and the *ser-4* gene also responded to hypoxia. Hypoxic regulation of serotonin receptors in different organisms will be investigated by identifying HIF-1 binding sites in the promoter regions.

Keywords: HTR1B, ser-4, HUVEC, C elegans, Hypoxia

P089
Combination of LDHA Inhibitor FX-11 with Tyrosine Kinase Inhibitor Masitinib Shows Synergistic Effects in KRAS-Mutant Cancer Cells

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Background/Aim: Activation of the *KRAS* oncogene is a key event in several types of malignancies, and recent studies have highlighted the role of KRAS in the metabolic reprogramming of tumor cells. Mutagenic activation of KRAS induces the expression of the lactate dehydrogenase A (LDHA) enzyme, which is essential for the glycolytic phenotype induced by KRAS. Although LDHA targeting alone exhibits antiproliferative and antitumorogenic effects, metabolic redundancy and plasticity associated with KRAS hyperactivation may limit the potential usefulness derived from targeting a single metabolic enzyme. We therefore conducted a pharmacological screening of an anticancer drug library to identify therapeutics that synergize with LDHA inhibition in killing KRAS-mutant tumor cells.

Material and Method: To inhibit LDHA, a potent small-molecule inhibitor called FX-11 was used. For single and combinatorial screening, a library of 1143 FDA-approved anticancer drugs was utilized. Screening was performed on HCT116, a KRAS-mutant colon cancer cell line. Cell viability was assessed by the Sulforhodamine B (SRB) assay. Synergistic combinations were also tested in PANC-1 and MIA PaCa2 KRAS-mutant pancreatic adenocarcinoma cell lines. The online SynergyFinder program was used to perform synergy calculations.

Results: A total of eight drugs exhibited higher antiproliferative effects in HCT116 cells when combined with FX-11 than each drug alone. Among these, the combination of LDHA inhibition with masitinib, a tyrosine kinase inhibitor, at various concentrations showed significant synergistic effects. Specifically, the combination index (CI) values that were lower than 1, indicating synergy, were as follows: 0.7 for 20 μM masitinib/10 μM FX-11 in HCT-116 cells; 0.95 for 20 μM masitinib/10 μM FX-11 and 0.8 for 20 μM masitinib/5 μM FX-11 in Mia-Pa-Ca2 cells; and 0.6 for 20 μM masitinib/10 μM FX-11 and 0.47 for 20 μM masitinib/5 μM FX-11 in PANC-1 cells.

Conclusion: These findings suggest that targeting LDHA via FX-11, in combination with masitinib, offers a promising therapeutic strategy for KRAS-mutant cancers.

Keywords: KRAS, LDHA, FX-11, masitinib

P090
Investigating the Cytotoxic Effects of Aurora Kinase B Inhibitor BI831266 Alone and in Combination with Cisplatin on DLD-1 Colorectal Cancer Cells

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Aurora kinases (AURKs), part of a highly conserved family of serine/threonine kinases, play critical roles in regulating the cell cycle. In humans, this family comprises three members: Aurora kinase A, B, and C, which serve as key mitotic regulators essential for maintaining chromosome stability. Overexpression and/or gene amplifications of AURKs have been demonstrated in various human cancers, including colorectal carcinoma (CRC). Among the different AURKs, there is a strong correlation between AURKB overexpression and clinicopathological parameters, as well as overall survival rates in CRC. Therefore, AURKB could be used as a promising biomarker and a potential therapeutic target for CRC.

In this study, we aimed to investigate the effect of BI831266, a selective AURKB inhibitor, alone (0-1-5-10-50-100 nM) and in combination with cisplatin (0-2.5-5-10-25-50 uM), on the cell viability and colony-forming capacity of DLD-1 colorectal cancer cells. To this end, we applied crystal violet cell viability and clonogenic assays, respectively. The combination indexes (CIs) were determined from cell proliferation data using CompuSyn software.

The results revealed that BI831266 suppressed cell proliferation and colony formation capacity of DLD-1 cells, both alone and in combination with cisplatin, in a concentration-dependent manner. The IC_{50} values from clonogenic assays showed that BI831266 was more effective both alone and in combination with cisplatin, compared to cisplatin alone. Similarly, in the crystal violet cell viability assay, BI831266 demonstrated enhanced efficacy, especially when combined with cisplatin, indicating a synergistic effect in both assays.

These findings, for the first time, demonstrate that the selective AURKB inhibitor BI831266 and cisplatin combination treatment synergistically inhibit the viability and colony formation capacity of DLD-1 cells at specific doses, suggesting it could be evaluated as a promising therapeutic strategy for cancer treatment. Further studies are needed to elucidate the underlying mechanisms and optimize the application of this combination therapy.

Keywords: Aurora Kinase B, Cell Cycle Inhibitors, Colorectal Cancer, BI 831266

P091
Lovastin Synergizes with GOT1 inhibitor to Kill KRAS-Mutant Cancer Cells

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Background/Aim: Glutamate Oxaloacetate Transaminase 1 (GOT1) is a metabolic enzyme involved in the aspartate-malate shuttle, glutamine metabolism, and the Krebs cycle. GOT1 has been shown to be expressed in cancers, and plays a critical role in KRAS-induced metabolic rewiring. Targeting GOT1 alone has antiproliferative and antitumorogenic effects; however, the potential utility of targeting a single metabolic enzyme may be limited by metabolic redundancy and plasticity associated with KRAS hyperactivation. This study aims to screen a large scale anticancer drug library in combination with GOT1 inhibitor-1 to identify novel synergistic combinations that show efficacy in KRAS-mutant cancer cells.

Materials and Methods: To inhibit GOT1, a potent, tryptamine-based derivative small molecule inhibitor called GOT1 inhibitor-1 was used. For screening, an FDA-approved anticancer drug library containing 1143 drugs was employed. Screening was performed on the KRAS mutant colorectal cell line HCT116. Cell viability was assessed by the Sulforhodamine B (SRB) assay. Promising combinations were selected for synergy testing using CellTiter-Glo assay. The synergistic effects of Lovastatin, an inhibitor of the 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) enzyme, and GOT1 inhibitor-1 were further tested on KRAS-mutant pancreatic adenocarcinoma cell lines PANC-1 and MIA PaCa-2, and immortalized pancreatic duct cells (HPNE) and their KRASG12D-transformed counterparts (HPNE/KRAS). The online SynergyFinder program was used to perform synergy calculations.

Results: The combined use of lovastatin, a cholesterol lowering drug, with GOT1 inhibitor-1 exhibited a
significantly greater anti-proliferative effect on HCT116, PANC-1, and MIA PaCa-2 cell lines than either inhibitor alone. We further showed that this combination showed profound synergy based on various sygergy models such as Bliss independence. Importantly, lovastatin/GOT1 inhibitor-1 combination was more potent in reducing the proliferation of HPNE/KRAS cells, compared with HPNE cells, suggesting a metabolic vulnerability involving HMGCR and GOT1 enzymes induced by KRAS activation.

Conclusion: The combination of GOT1 inhibitor-1 with Lovastatin demonstrates a promising therapeutic strategy for targeting KRAS-mutant cancer cells.

Keywords: KRAS, GOT1, Lovastatin

P092
miR-27a-5p Functions As a Tumor Suppressor Through Regulating STAT3 in Prostate Cancer

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Introduction and Aim: miR-27a-5p has been shown to act as a tumor suppressor miRNA in prostate cancer. The regulatory relationship between miR-27a-5p and the EGFR/Akt/mTOR signaling pathway has been revealed; however, the other pathways regulated by this miRNA have yet to be fully elucidated. We aimed to demonstrate the tumor suppressor role of miR-27a-5p by regulating STAT3, a well-known oncogene in prostate cancer.

Materials and Methods: The binding potential of miR-27a-5p to 3' UTR of STAT3 mRNA was identified *in silico*. The expression level of STAT3 was determined in DU145 cells transfected with miR-27a-5p mimics and inhibitors. The effect of miR-27a-5p on cell viability and migration of prostate cancer cells was elucidated by CVDK-8 and wound-healing assay.

Results: miR-27a-5p mimics were found to significantly downregulate the expression of STAT3, while suppression of miR-27a-5p led to increased expression of STAT3 in DU145 cells. Elevated expression of miR-27a-5p was able to significantly reduce cell viability in DU145 cells; whereas, it did not lead to any significant change in cell viability of PC3 cells. Migration of DU145 cells was suppressed with increased miR-27a-5p levels.
Conclusion: We showed that miR-27a-5p functions as a tumor suppressor through negatively regulating oncogenic STAT3 signaling in STAT3-positive DU145 cells. It was found that miR-27a-5p is not able to suppress cell viability of STAT3-negative PC3 cells, highlighting that the tumor suppressor function of miR-27a-5p is dependent on STAT3.

Keywords: miRNA, Prostate Cancer, STAT3, DU145

P093
Investigating Novel Mechanisms Regulating Myoblast Fusion and Skeletal Muscle Regeneration

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Background/Aim: Skeletal muscle regeneration is a complex process involving the fusion of myoblasts into multinucleated myofibers. This study aims to understand the roles of novel mechanisms in myoblast fusion and skeletal muscle regeneration, using both in vitro and in vivo models.

Materials and Methods: We utilize primary mouse myoblast cells to study the fusion of these cells into myotubes. Upon treatment of a differentiation-inducing medium, myoblast cells fuse to form elongated myotubes. To validate our system, we overexpressed a well-known fusogenic gene myomixer (Mymx) and suppressed its expression using RNA interference. Adenoviral vectors were generated for Mymx overexpression and knockdown. Myotube cultures were stained with a myosin heavy chain (MyHC) antibody and DAPI. As an in vivo model, we utilized a muscle injury model. The tibialis anterior (TA) muscle of mice is injured using cardiotoxin (CTX), and regeneration was assessed on days 10 and 14 post-injury. TA muscles harvested from these mice are stained with hematoxylin and eosin (H&E) or laminin and DAPI. Quantification included counting nuclei with centrally located positions and measuring cross-sectional areas of muscle fibers to assess the extent of regeneration.

Results: Mymx overexpression in myoblast cells significantly induced the fusion of these cells into myotubes while the depletion of Mymx negatively influenced this process. Post-CTX injury, expression of myogenic and fusion genes including Mymx were induced. We detected lesions in muscle tissue 3 days post-injury. However, regenerating myofibers characterised by centrally located were detected after 10-14 days. In the future, we will use the myotube culture and muscle injury models to delineate the roles of genes of interest in myogenic fusion and muscle regeneration.

Conclusion: Our studies will shed light on the novel regulatory mechanisms in skeletal muscle regeneration and underscore the importance of novel players in myoblast fusion and muscle repair. Further research will elucidate the precise molecular interactions and potential clinical applications.

Keywords: myoblast fusion, skeletal muscle regeneration, cardiotoxin injury

P094
Mutational Signature Detection in Whole Exome Sequencing: Insights from Esophageal Squamous-Cell Carcinoma

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Mutational signatures are patterns of mutations associated with biological processes such as DNA damage, repair mechanisms, and environmental exposures. These signatures hold great potential for clinical applications, including early cancer detection, targeted therapies, and predicting treatment responses. While whole genome sequencing (WGS) has successfully identified these signatures and shown promising clinical results, whole exome sequencing (WES) has limited detection power due to the lower number of mutations detected. Considering the affordability and interpretability of WES in clinical settings, there is a need to develop methods to accurately determine mutational signatures from WES data. Our study analyzed 552 esophageal squamous-cell carcinoma WGS samples and corresponding down-sampled WES data. Our results revealed that the detection power of WES was reduced by half for all signature types, including SBS, ID, and DBS. These initial findings suggest that further development, including the application of deep learning models, is necessary to enhance the sensitivity and accuracy of mutational signature detection in WES data, making it a valuable tool for clinical applications.

Keywords: mutational signatures, bioinformatics, genomics, cancer genomics

P095
Crosstalk Between Circadian Rhythm and Aryl Hydrocarbon Receptor (AhR) Signaling: Chronotoxicology Targets in Human Bone Marrow Adipose Tissue MSCs

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Background/Aim:
Human bone marrow adipose tissue-derived mesenchymal stem cells (hBMAT-MSCs) are vulnerable to xenobiotic-induced toxicity, such as environmental toxins or drugs. Literature highlights reciprocal interactions between Aryl hydrocarbon Receptor (AhR) signaling, also known as the xenobiotic receptor, and circadian rhythm. Circadian rhythms strongly regulate xenobiotic detoxification metabolism. This study aimed to investigate mechanisms of drug-resistant stroma that could be targeted to alleviate the treatment burden on the bone marrow.

Materials and Methods:
Bone marrow samples from three healthy donors were collected. BMAT-MSCs were isolated and expanded up to passage 3 and then were synchronized with Dexamethasone (100 nM, one-hour) and treated with 100 nM 6-Formylindolo[3,2-b]carbazole (FICZ, AhR-agonist) or 1.5 µM StemRegenin1 (SR1, AhR-antagonist). RNA was extracted at Zeitgeber time (ZT) ZT6 (day) and ZT18 (night) and analyzed using RT-qPCR.

Results:
Circadian-controlled expression of 15 genes was observed across all treatments, including clock genes (BMAL1, CLOCK, NPAS2, CRY1/2, PER1-2), AhR target genes (AHR, AHRR, ARNT), and detoxification genes (CYP1A1/B1, NRF2, GSTT2B, ABCA1). FICZ increased CYP1A1/B1 expression, potentially disrupting circadian gene expression. SR1 treatment abolished diurnal variation in CYP1A1 expression, suggesting the involvement of BMAL1, CLOCK, and CRY2 in circadian regulation. Phase shift (advance) was observed following SR1 treatment. The untreated group exhibited high expression of the GSTT2B gene at ZT6, followed by decreased expression at ZT18. Following FICZ and SR1 treatments, FICZ reduced GSTT2B expression at ZT6, while SR1 significantly increased it. SR1 decreased ABCA1 expression, whereas FICZ increased it at ZT6. These findings indicate that AhR ligands can modulate circadian manner detoxification gene expression.

Conclusions:
Exploring the circadian regulation of xenobiotic detoxification indicated time- and dose-dependent drug effects. Phase advances may identify molecular targets in circadian rhythm, aiding targeted chronotoxicology treatments. Consequently, the crosstalk between circadian rhythm and AhR signaling may be a primary target in the field of chronotoxicology.
This work was supported by the TUBITAK (Grant no: 220S759).

Keywords: bone marrow adipose tissue, mesenchymal stem cells, circadian rhythm, AhR signaling

P096
Disruption of Circadian Rhythm and Its Epigenetic Transmission to Offspring in terms of Brain Aging Acceleration

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Aim: Overdose of Methylphenidate can cause sleep problems, that can disrupt the circadian rhythm. Chronic exposure to Methylphenidate leads to neurobehavioral adaptations that persist into adulthood in zebrafish regardless of sex. Suggesting that the misuse of Methylphenidate during adolescence can alter neurobehavioral plasticity and these alterations might persist until adulthood. So usage of Methylphenidate in adult can not be compansated and have long term consequences in the egg they produce. Thus, harmful effects can be transmitted to offspring epigenetically according to our hypothesis in terms of accelerated brain aging in the second generation.

Materials and Methods: Wild Type (AB) zebrafish is utilized in this project. In the first generation of zebrafish, there are four different experimental groups. the first group only takes the methylphenidate to see its effect on circadian rhythm disruption. The second experimental group takes methylphenidate and oxytocin hormone. The third group takes methylphenidate and melatonin hormone. The last group is the control group and it does not take any drug. After the completion of treatment, breeding setup is put for experimental groups. The embryos from all four experimental groups are raised to 5 dpf as a second generation. DNA isolation is performed for 5 dpf embryos for methylation analyses and relative telomere length analysis. RNA isolation and behavioral experiments are done for first generation.

Results: It is an ongoing project and it is found that disruption of circadian rhythm via Methylphenidate can lead to changes in relative telomere length and oxytocin hormone has a compensatory effect. Other results would be reflected in the poster presentation.

Conclusion: Methylphenidate overdose can lead to circadian rhythm disruptions and it is dependent on the dopamine pathway. Different treatments are utilized in that project to understand the mechanism between dopaminergic and oxytocin pathway in terms of circadian rhythm and brain aging mechanism.

Keywords: Zebrafish, Brain Aging, Longitudinal, Circadian Rhythm

P097
Clinical And Molecular Findings In Two Moroccan Cases With Krabbe Disease

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Background: Krabbe disease, also known as globoid cells leukodystrophy (OMIM 245200), is a rare inherited neurodegenerative disorder characterized by severe demyelination in the central and peripheral nervous systems due to deficiency of the lysosomal enzyme galactocerebrosidase, encoded by the *GALC* gene located on chromosome 14q31. Clinical manifestations typically begin in early infancy covering symptoms such as hyperirritability, unexplained fever, feeding difficulties, psychomotor regression, spasticity. As the disease progress, it leads to severe neurological decline, including vision and hearing loss, paralysis, and death before the age of two. We report clinical and molecular findings of two Moroccan patients with this devastating condition.

Materials and Methods: Case 1: A 26-month-old girl from a healthy consanguineous couple, with a deceased sister who exhibited similar symptoms. At the age of 6 months, the proband presented psychomotor regression, unexplained fever, incessant crying, feeding difficulties, generalized hypertonia, blindness and deafness, leading to death at 2 years and 3 months.
Case 2: A 9-month-old girl, first child of a healthy non-consanguineous couple, presented with psychomotor regression at 4 months, fever episodes, incessant crying, feeding difficulties, hypertonia, and persistent snoring. She died at 1 year and 6 months.
Results: Case 1: Post-mortem exome sequencing of the proband revealed a homozygous pathogenic *GALC*
variant.Case 2: Whole exome sequencing (WES) identified the proband as compound heterozygous for two *GALC* pathogenic variants.Segregation analysis for parents is planned. Genetic counseling was provided to the families, with options for preimplantation or prenatal diagnosis for future pregnancies.

Conclusion:The identification of *GALC* variants highlights the crucial role of the geneticist in diagnosing Krabbe disease and providing families with genetic counseling, aiming to reduce recurrence risks. Though more common in consanguineous couples, our study emphasizes the importance of considering Krabbe disease in non-consanguineous couples as well, and raising awareness among health professionals to promote early diagnosis and better management strategies.
Keywords: Krabbe disease, GALC gene, psychomotor regression, genetic counseling, WES

P098
High-throughput analysis of membrane fluidity unveils a hidden dimension in immune cell states

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Cell membranes adapt to their surroundings and fulfill specific biological roles through biophysical alterations. However, the significance of such adaptations in human immune systems remains unclear, primarily due to the lack in proper methodologies. Here, we introduce a novel cytometry-based approach with single-cell resolution and multi-dimensional capability to address this gap. By integrating biophysical profiling with conventional biomarker analysis, this platform unveils distinct remodeling of plasma membrane fluidity across various immune cell types during immune responses and disease states. From in vitro studies on NK and T cells, we observed how membrane fluidity changes during immune response in a stimulus-dependent manner. This evidence strongly highlights the importance of investigating the fluidity remodelling as a means of cell adaptation/response to the environment. Indeed, by modifying membrane order, immune cells might increase their survival within the tumor microenvironment, enhance their migratory capacity and effector activity as well as the immune synapses integrity, thus affecting the overall immune responsiveness. From studies on patients with long COVID and chronic lymphocytic leukemia, we demonstrated that changes in membrane fluidity occur independently of surface marker expression. Furthermore, this biophysical parameter reveals novel functional and pathological immune cell states not apparent through surface marker profiling alone, and it can be used to sort cell population with distinct immune activities. Our discoveries offer insight into immune cell states based on their biophysical characteristics and enhance our comprehension of immune cell functional diversity. Since changes in membrane fluidity might generate as the result of multiple converging processes triggered by both physical stresses and biological stimuli, measuring the fluidity of the plasma membrane could be a straightforward approach to detect pathological states in cells, allowing diagnostic sensitivity without the need to delve into the underlying complexity of the system under examination.

Keywords: flow cytometry, membrane fluidity, inflammation, long COVID, leukemia, immunophenotyping, cell sorting

P099
Carbon Nanotube Based Delivery of β-glucuronidase (GUS) Reporter Gene to Brachypodium distachyon

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Background:
Plant transformation is a key tool in genetic studies, however, overcoming the plant cell wall barrier remains a major challenge. In order to overcome the drawbacks of the conventional transformation methods, novel gene delivery methods that are more efficient and independent of plant species and tissue remain to be investigated. Using nano particles (NPs) for transporting molecules in animal systems has been a fast-developing tool whereas it has shown slower development with plant systems. Single Walled Carbon Nano Tubes (SWCNTs) has been outstanding with their structural properties of high aspect ratio and tensile force which let SWNTs pass through the cell wall like a needle. In this study, SWCNTs were utilized for *Brachypodium distachyon*
callus transformation. The *β-glucuronidase* (GUS) gene was selected as a reporter for the optimization carbon nano tube based gene delivery process, allowing visual assessment of gene expression.

Materials and Methods:
Electrostatic grafting was used for loading the plasmids on SWNTs by creating a covalent conjugation between polyethylenimine (PEI), and COOH-SWNTs. The surface charge density was measured by Zetasizer Nano NS for confirmation of PEI attachment and determined to be within range before continuing. A 3:1 PEISWNT:DNA ratio was used for loading the reporter plasmid pCAMBIA1301, carrying the GUS gene controlled by CaMV 35S promoter, onto the PEI-SWNTs. Histochemical GUS Staining method was used for detection of transient expression.

Results:
The GUS gene products produced blue spots at the site of enzyme activity when the callus was incubated with X-gluc substrate. Transient gene expression served as an indicator for successful SWCNTs diffusion across intact cell membranes.

Conclusion:
SWCNTs may be beneficial given their ability of passive diffusion across cell walls and membranes which provides a genotype independent way for plant transformation. The findings demonstrate that SWCNTs can successfully carry reporter plasmids into
Brachypodium distachyon callus cells.

Keywords: SWCNTs, Brachypodium distachyon, Callus, Plant Transformation, Beta-glucuronidase

P100
Comparison of Serotonin-Related Human SLC6A4 and Caenorhabditis elegans MOD-5 Genes with Bioinformatics Tools

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The SLC6A4 gene in humans encodes the serotonin transporter protein known as 5-hydroxytryptamine (5-HTT). It has been shown that a polymorphism in the promoter of the SLC6A4 gene affects serotonin uptake rate and plays a role in sudden infant death syndrome, Alzheimer's patients with aggressive behavior, post-traumatic stress disorder, and depression susceptibility in individuals experiencing emotional trauma.
When examining the effects of serotonin signaling on cancer, numerous studies have demonstrated that serotonin stimulates cancer cell proliferation and inhibits apoptosis, thereby supporting tumor progression through the MAPK and PI3K/Akt signaling pathways.
In our study, the human SLC6A4 gene and its ortholog in *Caenorhabditis elegans*, MOD-5 genes were investigated. MOD-5 gene activates serotonin sodium symporter activity in *C. elegans* and plays a role in serotonin uptake. Our research aims to determine the similarity between the human SLC6A4 gene and the C. *elegans* MOD-5 gene using bioinformatics tools and to choose an appropriate model for a more detailed study of their effects on cancer.
In our research, various bioinformatics tools such as Bioedit, NCBI Blast, NCBI Blastn, PDB, Uniprot, OligoDT Analyzer, and Primer 3 were used. Initially, regulatory promoter sequences and gene sequences were obtained from NCBI and EPD The Eukaryotic Promoter Database. The promoter regions were analyzed separately, and the promoter regions of the selected genes from the two models were compared in terms of binding sites for different transcription factors.
As a result, the gene sequence similarity between the two genes was found to be % 74, and they exhibited an 85% similarity in amino acid composition. The highest similarity was identified between the 250 bp to 2840 bp regions. When the selected sequences of the promoter regions were compared, similarities were also observed. Considering all these results, it was concluded that *C. elegans* is a suitable model for detailed analyses of cancer related serotonin signalling pathway.

Keywords: SLC6A4, MOD-5, serotonin, 5-HTT, bioinformatics analyses

P101
EDA2R-NIK signaling and exercise-dependent therapeutic approaches

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Background: Cancer cachexia poses a complex and severe challenge, compromising patient well-being and treatment tolerability Although exercise holds potential as a remedy for alleviating cachexia, its viability is constrained for a substantial patient subset. Therefore, molecular pathway research for exercise-induced signaling pathways is required to explore novel pharmacological intervention targets. EDA2R, and its downstream mediator, NIK, have been previously reported as cachexia-inducing genes.

Methods: WT and NIK knockout male mice underwent an 8-week involuntary treadmill running as a part of a high-intensity interval training (HIIT) program. Before last training week, mice were placed in metabolic cages for analysis. After finalizing the training, grip strength and inverted screen tests were performed pre-sacrifice, followed by qPCR analyses of PGC1α, IL-6, and FOXO1 in cDNA samples isolated from gastrocnemius muscles.

Result: Our findings indicate the efficacy of the High-Intensity-Interval Training (HIIT) model in inducing metabolic changes both in WT and EDA2R-KO mice. Hypertrophy was lacking in muscle-specific NIK knockout (KO) mice compared to WT as evidenced by grip-strength and inverted screen tests. The expression of exercise-dependent genes (FOXO1, PGC1a, IL-6) showed favorable regulation in the HIIT group, with less pronounced effects observed in the EDA2R-KO mice.

Future Perspective: In the context of future perspectives, targeting EDA2R signaling is proposed for developing novel drugs for simulating the effect of exercise.

Conclusion: HIIT enhanced grip strength, indicating possible muscle hypertrophy. Exploring links between exercise markers and EDA2R-NIK signaling may unveil valuable drug targets.

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Keywords: Hypertrophy, Exercise, Non-canonical NFKB Signaling, Skeletal Muscle, High Intensity Interval Training

P102
Investigating the Mechanism of OTA-Induced eIF2α Phosphorylation and Global Translation Inhibition

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Ochratoxin A (OTA) is a mycotoxin produced by some *Penicillium* and *Aspergillus* genera of fungi, known for its genotoxic, hepatotoxic, immunotoxic, nephrotoxic, and carcinogenic effects. OTA induces eIF2α phosphorylation, a key event for inhibiting 5'cap-dependent translation initiation. OTA-induced oxidative stress, ER stress, and possible empty tRNA accumulation through Phe-tRNA synthetase inhibition may activate eIF2α kinases. While high dosages of OTA are known to inhibit protein synthesis, its effects on global translation rate and eIF2α phosphorylation are not fully explored. Preliminary studies in our laboratory show a time- and dose-dependent effects of OTA on eIF2α phosphorylation and global translation rates. This study aims to uncover the mechanism(s) involved in activation of eIF2α kinases and decreased global translation rate under OTA treatment.

Specific inhibitors A92, C16, and GSK2606414 were used to identify and investigate the possible roles of GCN2, PKR, and PERK, respectively, in OTA-induced eIF2α phosphorylation and global translation inhibition. Human kidney proximal tubule epithelial (HK-2) and rat liver progenitor (WB-F344) cell lines in were co-treated with these inhibitors and OTA in a dose-dependent manner. Changes in eIF2α, ERK, and Akt phosphorylation levels were assessed via Western blotting analysis, and translation rates were measured using the SUnSET (SUrface SEnsing of Translation) method with anti-puromycin antibody.

The results suggest that GCN2 and PKR kinases are possibly involved in OTA-induced eIF2α phosphorylation and translation inhibition at certain doses, although inhibiting neither completely abolished the OTA effects. Inhibition of both kinases decreased Akt and mTOR activation, indicating possible cross-signaling, while PERK inhibition showed no notable effects.

Our preliminary findings are shedding light on mechanisms of OTA toxicity in HK-2 and WB-F344 cell lines; specifically, how OTA affects global translation through eIF2α phosphorylation. Time-dependent experiments with the most effective doses will be conducted for further insights. Moreover, each kinase will be knocked down using shRNA to further elucidate their individual contributions.

Keywords: Ochratoxin A OTA, Global Translation, eIF2¿½

P103
Effect of Estrogen Receptor Signaling on Long Non-coding RNAs in MCF7 Breast Cancer Cells

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Background/Aim: Estrogen receptor (ER) signalling is crucial in cellular regulation, influencing coding and non-coding gene expression. Long non-coding RNAs (lncRNAs) are pivotal in cancer biology, affecting gene expression and cellular processes like proliferation and metastasis. Despite the limited number of known associations, ER's impact on lncRNAs remains underexplored in breast cancer. This study examines the time-dependent effects of estradiol induction on the lncRNA transcriptome in ER-positive MCF7 cells, aiming to detect differential expression and transcript isoforms.

Materials and Methods: Direct RNA sequencing assessed the impact of estradiol on the non-coding transcriptome, analyzing differential expression and transcript isoforms. Reporter assays evaluated cis- and trans-regulation of lncRNA transcription. Functional assays tested the roles of *SNHG9* in MCF7.

Results: We detected estradiol-induced differential gene and transcript expressions in various non-coding genes. Among the ncRNAs, 19 members of the Small Nucleolar RNA Host Gene (SNHG) family were affected by estradiol induction. *SNHG9*, known for its oncogenic features in pancreatic cancer, glioblastoma, and ovarian cancer, showed consistently increased expression with estradiol treatment. Functional assays demonstrated that *SNHG9* overexpression in ER-positive MCF7 cells led to higher colony formation and proliferation, while no such effect was observed in the ER-negative cell line (MDA-MB-231), suggesting a link to estrogen signalling pathways. Luciferase reporter assays indicated that *SNHG9* has multiple potential miRNA binding regions, with varying responses, indicating microRNA sponge function.

Conclusion: Our findings suggest the involvement of *SNHG9* as a microRNA sponge in breast cancer oncogenesis via estrogen signalling pathways. The shared regulatory elements in SNHG promoters hint at a common regulation mechanism by estrogen. Further investigations are needed to elucidate specific binding regions and regulatory mechanisms. This study provides insights into how estradiol affects and interacts with the SNHGs in breast cancers.

Keywords: lncRNAs, SNHG9, breast cancer, Estrogen receptor signalling

P104
A Transcript Variant; Playing Role in Drug Sensitization in Hela Cells

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Background/Aim: Apoptosis, a form of programmed cell death, is a cellular process that plays critical roles in the maintenance of cellular homeostasis. As apoptosis is crucial for controlling the balance between cell death and cell proliferation, inappropriate apoptosis can lead to various conditions, including cancer. Apoptosis is regulated by various proteins and genes within the cell. Additionally, apoptotic pathways can be induced by some molecules such as cisplatin, a chemotherapeutic agent that activates the intrinsic pathway of the apoptosis. The aim of this study is to characterize transcripts that play a role in regulation of apoptosis under cisplatin-mediated apoptotic conditions.

Materials and Methods: Firstly, total RNA, polyA + and polyA-eliminated fractions from cisplatin- and DMSO-treated HeLa cells were subjected to RNA sequencing to identify differentially expressed candidate transcripts. 5' and 3' RACE PCRs were performed to map the transcriptional units of candidate genes. The differential expression of candidates was confirmed by qPCR analysis. To characterize phenotypic effect, candidate gene was transiently silenced by siRNA transfection, and apoptosis rate was measured by flow cytometry analysis.

Results: The deep sequencing profile showed that a transcript variant of a candidate gene is differentially expressed under cisplatin conditions. Interestingly, we found that the transcriptional unit of the transcript variant is longer than its annotated version in the human reference genome assembly. Although the knockdown of the transcript variant does not cause a significant change in apoptosis rate, its knockdown combined with cisplatin treatment sensitizes the cells to cisplatin-mediated apoptosis, resulting in a higher rate of apoptosis.

Conclusion: The length of transcriptional unit of the transcript variant has been correctly determined. Even if the transcript variant does not directly lead to an apoptotic profile, it has the potential to play a role in drug sensitization in HeLa cells.

Keywords: apoptosis, drug sensitization, cisplatin, HeLa

P105
Exploring RNA Methylation Dynamics: Insights from FTIR and CD Spectroscopy

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Background/Aim: RNA modification involves chemical alterations during transcription that regulate RNA stability, localization, and function, impacting health and disease. Despite costly and complex methods for RNA methylation analysis at specific sites or across the transcriptome, biophysical approaches remain underexplored, necessitating practical alternatives. Fourier transformed-infrared (FT-IR) and circular dichroism (CD) spectroscopy effectively differentiate methylated from unmethylated cellular RNAs and reveal distinct structures induced by m^1A and m^6A methylation, offering viable, cost-effective tools for studying RNA methylation and biophysical properties.

Methods: We utilized FTIR spectroscopy to examine the methylation levels in total RNA from HeLa cells. By knocking down METTL3 and TRMT61A, enzymes responsible for m^6A and m^1A methylation respectively, we confirmed reduced methylation levels. Total m^6A RNA methylation in both control and knockdown cells was quantified using a colorimetric assay and an FTIR spectrometer (Perkin Elmer, UATR2). Additionally, the secondary structures of HeLa cell-derived total RNAs were analyzed using a CD spectrometer (Jasco J-1500). We explored whether these methods could assess changes in RNA methylation marks following extracellular stimuli like tumor necrosis factor α (TNF-α), which induces the extrinsic apoptotic pathway in HeLa cells, revealing alterations in the cellular RNA methylome under various conditions.

Results: Differences in methylation were detected between treated and untreated cells in the 3500-800 cm-1 IR region using the FTIR spectrometer. CD data of HeLa cell-derived total RNA samples exhibited absorbance differences in the CD spectrum, likely due to changes in the chirality of RNA molecules upon methylation. Our protocol demonstrates that these methods can be employed to assess perturbations in methylation marks upon extracellular stimuli in eukaryotic cells.

Conclusions: FTIR and CD spectroscopy are effective, cost-efficient methods for analyzing RNA methylation and its biophysical properties, enabling the detection of methylation changes and perturbations in response to extracellular stimuli like TNF-α in eukaryotic cells.

Keywords: RNA methylation, N6-methyladenosine, FTIR spectroscopy, CD spectroscopy

P106
Recombinant TAT-SMN1 Fusion Protein produced to treat SMA disease

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Spinal Muscular Atrophy is a disease characterized by muscle weakness and atrophy caused by permanent deterioration of the anterior horn cells in the spinal cord. SMA is the most common genetic cause of infant mortality and is autosomal recessive. SMA is caused by defects in the SMN1 gene. Treatment strategies are very limited and very expensive in the world.

The aim of this study is to produce recombinant SMN1 protein. At the same time, our second goal is to produce the recombinant SMN1 protein in a way that creates a fusion with the TAT targeting sequence so that it can easily infiltrate into the cell.

Within this purpose, firstly, the full lenght of SMN1 gene was sub-cloned into pET-21a vector by restriction enzyme digestion from SMN1-pEGFP/N2 vector. This clone was named as SMN1-pET21-a. In the second stage, cell penetrating sequence (TAT sequence) were sub-cloned into SMN1-pET21a vector using BamHI and EcoRI restriction digestion strategy. This clone was named as TAT-SMN1-pET-21a. The recombinant TAT-SMN protein was purified with histidine tail from the protein lysate by Ni-NTA method. Purification was confirmed by SDS-PAGE and Western blotting. The activity of TAT-SMN1 recombinant protein was identified in SMN-1 silenced SH-SY5Y cells that previously generated in our another study. First, cytotoxic effect of recombinant TAT-SMN1 protein was performed with MTT assay. At the same time, immunofluorescence analysis was performed to demonstrate the entry of the recombinant TAT-SMN1 protein into the cell.

As a result of the study, the TAT-SMN1 gene was cloned into a prokaryotic expression vector, and the recombinant TAT-SMN1 fusion protein was produced and purified. Cytotoxicity analyses determined that the protein is not toxic to SMN1-silenced SH-SY5Y cells. Additionally, the ability of the TAT-SMN1 fusion protein to enter the cells was demonstrated using IFC analysis.

Keywords: SMA, Recombinant, TAT-SMN1

P107
Investigating the statin response on breast cancer spheroids and cancer-associated fibroblasts in changing stiffness environments

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Background/Aim: Breast cancer remains to be the most common cause of cancer-related death in women. It is now recognized that the structure, composition, and mechanical stiffness provide major microenvironmental cues that modulate tumorigenesis and tumor progression, as well as treatment response. In vitro preclinical studies are limited particularly for emerging therapeutics such as statins that have recently been repurposed as a potential cancer therapy. This study investigates matrix stiffness-based effects on statin-based breast cancer treatment response in breast cancer spheroids and breast cancer-based co-culture systems.

Materials and Methods: A 3D hydrogel cell culture system based on sodium alginate (SA) and rat tail collagen type 1 (RTC) was developed where hydrogel stiffness and cell-matrix adhesion were independently controlled by varying concentrations of SA and RTC between 1.5 – 30 mg/mL and 0 – 1.5 mg/mL, with a storage moduli of sodium alginate hydrogels were ranging from 0.03kPa to 3.6kPa. Growth of MCF-7 and MDA-MB-231 in hydrogels was monitored up to 14 days and quantified by brightfield and fluorescence microscopy analysis. The cytotoxic effects of atorvastatin on MDA-MB-231 in the lack/presence of human normal fibroblast (HMFU-19) or breast cancer-associated-fibroblast (CAF544) were quantified by IC_{50} values based on Alamar Blue assay.

Results
Spheroids larger than 3000µm^2 in 5 to 30 mg/mL SA hydrogels were measured, and the average spheroid sizes were 5415.74, 6054.95, 16668.32, and 66904.25 µm^2, respectively. The IC_{50} values of MDA-MB-231 are 1.88 µM, HMFU-19 is 17.18 µM, and their co-culture has a value of 25.82 µM while CAF544 has 21.29 µM and its co-culture with MDA-MB-231 has 19.52 µM IC_{50} values.

Conclusion
This study shows an association between increasing matrix stiffness and breast cancer spheroid size, as well as reporting the IC_{50} values of MDA-MB-231, HMFU-19, and CAF544 and their co-culture under 3D cell culture conditions via hydrogel systems.

Keywords: Matrix Stiffness, Statins, 3D Cell Culture, Breast Cancer

P108
Profiling the Proximity Interactome of Ki67 in a Cell Cycle Dependent Manner

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Background/aim: Ki67 is a widely used proliferation marker in oncology. Studies have shown that Ki67 plays role in heterochromotin organization, ribosome biogenesis and maintenance of mitotic chromosomes. However, the molecular function of Ki67 whose expression and localization change according to the cell cycle stage, has not been fully elucidated. Uncovering the proximity interactome of Ki67 is important in shedding light on the function of this protein in cell division. Because Ki67 is a very large protein (359 kDa), it is possible that N and C terminal specific protein networks show differences. Moreover, it is highly probable that the mitosis and interphase specific interaction networks of Ki67, which is located on the surface of condensed chromosomes in the mitotic phase and seen in the nucleolar region in the interphase, are distinct from each other. Our aim is to unravel the mitosis and interphase specific interaction networks of Ki67 at both N and C termini via miniTurbo proximity *in vivo* labeling method.

Materials and methods: Molecular cloning techniques were employed to generate miniTurbo tagged Ki67 expression vectors. HeLa Kyoto cells were transfected with the vectors and immunofluorescence staining was performed to confirm both the correct cellular localization of recombinant Ki67 and its biotinylation specificity. The biotinylation efficiency was analysed by Western Blotting.

Results: Immunofluorescence staining of HeLa Kyoto cells transfected with Ki67-miniTurbo-3xHA and 3xHA-miniTurbo-Ki67 vectors revealed that both recombinant Ki67 proteins localized to specifically around the nucleolus in interphase cells and around the chromosomal surface in mitotic cells. Biotinylation was specific and restricted to the Ki67's localization.

Conclusion: Our next steps will be to pull down the biotinylated proteins and to perform LC-MS/MS analysis to identify Ki67 interactomes at both N and C termini in interphase and mitosis. Addressing the roles of these interactions will help us to understand the molecular function of Ki67.

Keywords: Ki67, cell division, miniTurbo, proximity labeling

P109
Green Synthesis of Mesoporous Silica Nanoparticles for Medical Applications

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Background/Aim: Mesoporous Silica Nanoparticles (MSNs) are extensively utilized in various biomedical applications due to their exceptional properties. Conventional synthesis methods for MSNs typically involve high costs with expensive alkoxy silanes. Although sodium silicate presents a cost-effective alternative as a silica source, its preparation can involve harsh chemicals resulting with environmental pollution. Silica-rich bio-sources such as rice husk (RH) and horsetail plant stalk (HTS) are effective substitutes for chemically synthesized precursors, providing sustainable method for synthesizing well-structured MSNs. These agricultural wastes, which contain 10-25% silica by dry weight, offer an eco-friendly and economic way to convert low-value agricultural wastes into high-value nanomaterials.

Materials and method: In this study, we developed a plant-mediated green synthesis method for MSNs, leveraging the silica content of RH and HTS. The MSNs were synthesized through the hydrolysis and condensation of sodium silicate, derived from these bio-sources in the presence of surfactants. Results:

The resulting nanoparticles, designated as MSN-1 (from RH) and MSN-2 (from HTS), were characterized by FT-IR and XRD techniques, confirming the existence of pure silica networks. Further analysis using BET/BJH, and HR-TEM revealed mesoporous structure of MSN-1 and MSN-2, which exhibited high specific surface area and large pore volumes. HR-TEM images clearly showed the highly ordered mesoporous structure of these nanoparticles. The *in vitro* MTT assay was conducted on ECV304 cells treated with MSN-1 and MSN-2 at concentrations ranging from 1 to 800 µg/mL over 24, 48, and 72 hours. No toxicity was observed even at the highest concentrations, indicating the biocompatibility and biosafety of the synthesized nanoparticles.

Conclusion: These results demonstrate the potential of MSN-1 and MSN-2 as promising candidates for surface functionalization and biomedical applications, owing to their biocompatibility, eco-friendly synthesis, and super structural properties.

Keywords: Mesoporous Silica Nanoparticles, Green synthesis, Biocompatible

P110
Purification of ROS1 fusion proteins for non-Small Cell Lung Cancer Diagnostic and Treatment Strategies

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Background: The ROS1 fusion protein is found in lung cancer patients and is associated with aggressive, rapidly spreading cancer. Identifying ROS1 and its fusion genes, EZR-ROS1 and CD74-ROS1, is crucial for treatment strategies. These recombinant proteins are not commercially available but are essential for diagnostic pathology and drug research.

Aim: To investigate the mechanisms of ROS1 rearrangement in lung cancer, and to develop recombinant proteins that can be used to development of antibodies and drugs targeting ROS1 fusion proteins.

Methods: The pcDNA3.1 EZR-ROS1 and pcDNA3.1 CD74-ROS1 constructs were sub-cloned into E. coli DH10a and then transfected into the A549, Calu1, and Hek 293 cell lines. These cell lines were cultured in appropriate media to analyze the expression of CD74-ROS1 & EZR-ROS1 proteins using Western blotting. A nickel column was used for purification.

Results: Our findings revealed that Calu1 cells express significant quantities of recombinant proteins, EZR-ROS1 and CD74-ROS1. Moreover, we observed high levels of EZR-ROS1 and CD74-ROS1 expression in the transfected HEK 293 cells. Conversely, our analysis showed no detectable expression of EZR-ROS1 and CD74-ROS1 in the A549 cell line

Conclusion:
These results provide the groundwork for further research on ROS1 fusion proteins, which may be an effective treatment strategy for the subset of patients with NSCLC whose tumors express ROS1 fusion genes.

Keywords: ROS1 fusion, Lung cancer, protein purification

P111
Genome-wide Identification, Characterization and Correlation with Plant Stress of SAPs in Glycine max

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Aim: Glycine max (soybean) is a highly valued crop globally, both economically and agriculturally. However, the current climate crisis significantly affects agricultural productivity. The ability of plants to respond to environmental stress conditions is of paramount importance for agricultural yield. Stress-Associated Protein (SAP) proteins are a family of transcription factors playing a crucial role in enhancing stress tolerance in plants. This study aimed to perform a genome-wide identification and characterization of the SAP protein in soybean.

Materials and Methods: Genomic, coding, and protein sequences belonging to the SAP gene were downloaded from Plant TFDB and NCBI in fasta format. The genes' chromosomal locations, exon-intron maps, protein motifs, and 3D models, cis-regulatory elements and miRNA targets, and subcellular localizations were determined *in silico*.

Results: According to the obtained data, two genes belonging to the SAP family were identified in soybean. These genes are localized on chromosomes 2 and 14. Both genes have one major intron region. The proteins possess conserved zinc finger motifs. Various cis-regulatory elements associated with different stresses were found. These genes are localized in the chloroplast, mitochondria, nucleus, cytoplasm, and plastids, indicating extracellular localization.

Keywords: SAP, soybean, plant stress, Genome-wide

P112
Functional Characterization of circCLASP1 in HeLa Cells

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Background/aim: CLASP1 (Cytoplasmic Linker Associated Protein 1) is a gene that regulates microtubule dynamics and plays critical functions in cell migration and cytoskeleton structure. CLASP1 contributes to cancer biology in a variety of cancers, including ovarian and lung cancer. Li et al. utilizing a large-scale CRISPR-RfxCas13d method discovered that a circular RNA (circRNA) was transcribed at the Chr2:122363276-122363756 region of this gene. Although the existing studies show the involvement of circRNAs in apoptosis mediated by the chemotherapeutic drug cisplatin (CP), the expression pattern of circCLASP1 in the presence of CP remains unclear. The aim of this study is to determine the expression of circCLASP1 in CP-treated Hela cells and to investigate the effect of circCLASP1 on cell apoptosis and proliferation.

Materials and methods: HeLa cells were treated with CP. The expression level of circCLASP1 was determined using qPCR analysis. Following transfection with si-circCLASP1, circCLASP1 was silenced by 80%. The efficiency of silencing was also verified by qPCR. WST8 analysis was used to quantify proliferation, whereas flow cytometry with AnnexinV/7AAD was used to detect apoptosis.

Results: circCLASP1 expression was reduced by 2-fold in CP-treated Hela cells. circCLASP1 silencing decreased proliferation and increased apoptotic rate.

Conclusion: circCLASP1 expression level is modulated by CP, and with circRNA silencing, the cell undergoes apoptosis. In future research, the CP sensitivity impact will be studied by administering CP to circCLASP1 silenced cells.

Keywords: circCLASP1, cisplatin, apoptosis, HeLa

P113
Cloning and Characterization of the SMN2 Gene Promoter

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Background/Aim: Spinal Muscular Atrophy (SMA) is an autosomal recessive genetic disorder characterized by the degeneration of alpha motor neurons in the spinal cord, resulting in symmetrical muscle weakness. The SMN gene, spanning 20 kb and comprising 8 exons, encodes the 294-amino acid SMN protein. There are two copies of this gene: telomeric (SMN1) and centromeric (SMN2), differing by a 5-nucleotide variation in exon 7. In more than 95% of SMA patients, homozygous deletion of exon 7 in the SMN1 gene is observed. Despite being full-length, SMN2 typically produces a non-functional SMN protein due to alternative splicing. Research indicates that the number of SMN2 copies influences disease severity, prompting efforts to enhance SMN2 expression for therapeutic purposes. This study aims to characterize the SMN2 promoter region through detailed bioinformatic analysis and cloning.

Materials and Methods: The promoter region of SMN2 gene was identified using NCBI database tool. Bioinformatic analyses were performed to screen secondary structure, %GC content, and potential transcription factor binding sites of the SMN2 promoter. Specific primers were designed for PCR-based cloning into the pMetLuc reporter vector. Then the constructs were transiently transfected into Hep3B cells. Basal activities of promoter regions were assessed using Dual core Luciferase assay and SEAP systems.

Results: In a result of the bioinformatic analyses, the promoter region was consist of four GC islands and predicted transcription factor binding sites such as SP1, AP2, CEBP, and E2F. Fragments (888 bp, 618 bp, 457 bp, 223 bp, and 116 bp) of the SMN2 promoter were cloned into the pGEM-T Easy vector and subsequently sub-cloned into pMetLuc Reporter vector. Among these, the 223 bp fragment exhibited the highest basal activity.

Conclusion: This study is crucial for identifying strategies to activate the SMN2 gene through its promoter, potentially improving therapies for SMA.

Keywords: SMN2, bioinformatic, promoter, basal activity

P114
Promotor Region Analysis of All miRNAs Identified in Arabidopsis thaliana

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Bakground /Aim: MicroRNAs (miRNAs) are post-transcriptional regulators of gene expression in plants and play critical roles in many biological processes such as development and stress responses. miRNA expression is controlled by regulatory elements in their promoter regions. The aim of this study was to perform a comprehensive analysis of the promoter regions of all miRNAs identified in *Arabidopsis thaliana* to determine the characteristics and distribution of these regulatory elements.

Materials and methods: The 2 kb promoter regions preceding the gene start codon of *Arabidopsis thaliana*
miRNAs identified in miRBase and pmiREN databases were obtained by *in silico* methods. The cis-regulatory elements in the promoter regions were identified using the PlantCARE database and their distribution was analysed. In addition, potential transcription factor binding sites (TFBS) in promoter regions were identified.

Results: We demonstrated that various cis-regulatory elements and TFBSs are widely present in miRNA promoter regions in *Arabidopsis*. In particular, elements associated with abiotic and biotic stress responses (e.g. ABRE, W-box) and elements associated with developmental processes (e.g. TA-TA-box, CAAT-box) were frequently detected. Variations of these elements provide important clues on how miRNAs are regulated under different stress conditions and developmental stages.

Conclusion: Our results reveal that the expression of miRNAs in *Arabidopsis* is controlled by a complex regulatory network. The diversity of cis-regulatory elements and TFBSs in promoter regions helps us to understand how miRNAs can be modulated in response to different environmental and intrinsic signals. This study provides valuable information to better understand the mechanisms of epigenetic and transcriptional control of plant gene expression.

Key words: Cis-regulatory elements, *Arabidopsis thaliana*, promoter, miRNA

P115
Investigating the Roles of GPCRs in Ochratoxin A (OTA)-Induced Activation of MAPK/ERK1-2 Signaling Pathway

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Ochratoxin A (OTA) is a mycotoxin produced by fungi of the *Aspergillus* and *Penicillium* genera and is known to activate MAPK/ERK1-2 signaling pathway associated with cell proliferation, differentiation, migration, aging and apoptosis in various cell lines. However, how OTA activates MAPK/ERK1-2 pathway is not known. Our preliminary studies suggest that OTA may activate the MAPK/ERK1-2 pathway via a G protein-coupled receptor (GPCR). The aim of this study is to identify GPCR(s) involved in OTA mediated regulation of the MAPK/ERK1-2 signaling pathway. GPCR modulators were used to test whether OTA-induced MAPK/ERK1-2 activation depends on GPCRs. Then, a commercially available GPCR panel was used to screen the 168 receptors potentially activated by OTA. Additionally, samples were prepared for mass spectrometry analysis by performing TURBO-ID affinity biotin labeling assay to identify the candidate GPCRs that interact with β-arrestin in cells after exposure to OTA. Since it is known that GPCRs are desensitized after activation, endosomes were isolated upon OTA exposure and examined by mass spectrometry analysis.

As a result of screening of 168 GPCRs via GPCR panel, no OTA-activated GPCR was detected. However, after mass spectrometry analysis performed for both endosome isolation and TurboID proximity labeling experiment, it was observed that OTA induced both the biotinylation rate and their presence in endosomal fractions of the following GPCRs: GPR142, GABBR1, TAAR1, GPRC6A, CASR and HTR6.

In summary, GPCRs identified in this study may play a role in dysregulation of the MAPK/ERK1-2 signaling pathway by OTA, and hence, in the toxicity of OTA. Validating these receptors will be an important step in understanding the cellular effects and possible toxic mechanisms of OTA. This study lays an important foundation for future research aimed at understanding the biological effects of OTA and reducing OTA-induced toxicity.

Keywords: MAPKERK1-2, Ochratoxin A OTA, G protein-coupled receptor GPCR

P116
The Roles of Disordered Effector Domains on the Developmental Functions of Drosophila Melanogaster Transcription Factor Bicoid

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Background & Aim: Bicoid is a maternal transcription factor, coded by bcd gene, critical for early embryonic development. It forms an anterior to posterior (AP) gradient, which is important for the spatiotemporal regulation of more than fifty target genes involved in Drosophila segmentation. Bicoid contains a structured DNA binding homeodomain and disordered effector domains, that are involved in various functions critical for embryo survival. They are less conserved and do not naturally fold into a stable conformation; however might form various conformations upon DNA or protein interactions.

Bicoid effector domains are named based on the aminoacid content and biochemical features such as glutamine-rich, alanine-rich and acidic domains. What these effector domains with different chemical features do at different concentrations and hundreds of different targets of Bicoid is a question whose answer will help us understand Bicoid regulation and the role of disordered domains that make up over the third of human proteome.

Material and Methods: To investigate the role of transcription factor disordered effector domains, we are generating transgenic flies with truncated *bcd* alleles lacking some or all of the effector domains that are tagged with GFP using 2-step CRISPR-Cas9. In the first step, we generate *bcd* mutant lines with landing sites using CRISPR and in the second step we insert the tagged truncated constructs using recombination-mediated cassette exchange (RMCE).

Expected Results: We expect to see differential regulation of Bicoid targets along Bicoid concentration gradient and at different developmental times due to various interactions mediated by the effector domains. We will observe developmental and molecular effects by cuticle analyses of larvae and in situ hybridization of several target genes along the AP axis. We will also assess the effects of the truncations on Bicoid's genome-wide and along the embryo DNA binding specificity, co-factor interactions, transcriptional dynamics, and gradient formation via ChIP-seq, Mass-Spectrometry, and live and fixed imaging assays, respectively.

Keywords: Transcription, Development, Drosophila

P117
Dietary Polyphosphate Exposure and Its Effects on Neuroblastoma Metastasis Through Wnt/ROR1 Pathway Activation

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Background/Aim: Neuroblastoma is the most common extracranial solid tumor in high-income countries, accounting for approximately 7% of all childhood cancers, suggesting a potential link between diet and tumor development. Industrially processed foods, widely consumed in developed and developing countries contain higher levels of phosphates than natural foods. These high levels of phosphates, mainly from polyphosphates used as additives in industrial food production, disrupt the AKT signaling pathway and impair normal brain growth in young mice. This pathway is involved in the ROR1 receptor-mediated Wnt signaling pathway, which promotes metastasis by enhancing the EMT (epithelial-mesenchymal transition) characteristics of the cells. The aim of this study is to investigate the metastatic properties associated with the WNT/ROR1 signaling pathway in SH-SY5Y neuroblastoma cells exposed to high levels of inorganic phosphate.

Methods: To investigate phosphate accumulation in the SH-SY5Y neuroblastoma cell line, cells were treated with 5 mM NaH□PO□ and 25 µM sodium hexametaphosphate. Changes in signaling pathways were determined using Western blotting. A wound healing assay was performed to evaluate cell migration capacity.

Results: In our study mimicking a high-level Pi diet, we observed significant changes in the phosphorylation of proteins within the Wnt pathway (Phospho-AKT, Phospho-SRC) and the expression of its receptor ROR1 in SH-SY5Y cells. These alterations in Wnt signaling were associated with increased expression of EMT markers (N-cadherin, ETS1, SNAIL, ROCK2, TWIST1).

Conclusion: These results suggest that high polyphosphate exposure leads to the acquisition of metastatic properties in neuroblastoma cells through the ROR1 receptor and Wnt signaling pathway.

Keywords: Neuroblastoma, polyphosphate, WntROR1, EMT

P118
Unraveling the Consequences of Point Mutations: A Comparative Analysis of AlphaFold Predictions and Experimental Structures

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Background: Mutations in human proteins can arise from environmental or genetic factors, impacting protein behavior and functionality. These mutations can result in neutral or deleterious effects, altering protein dynamics and influencing their functional properties. Understanding the interplay between function, dynamics, and structure is crucial for grasping these effects.

AlphaFold (AF) algorithms have made a major advancement by accurately predicting protein structures using homologous protein structures and neural networks This study aims to assess the (AF) proficiency in predicting the structural and dynamic changes of proteins resulting from single, double, and triple point mutations. This will be achieved by comparing the dynamic alterations in AF-predicted structures for both the wild type and mutant proteins against experimentally determined structures.

Method: We employed the Gaussian Network Model (GNM) to analyze mutations, examining dynamic differences between wild-type and mutant structures using the experimental structures and AF predicted structures. In this study, 100 different wild type proteins with experimental mutant structures ranging from 1 to 100, 700 being in total. Residue fluctuations and correlation between residue fluctuations in slow modes of motion, and their degrees of collectivity are calculated to quantify differences between neutral and deleterious mutations.

Results: Our results show that deleterious mutations exhibit relatively more significant variations in hinge point, the dynamic profiles and correlations of residues, global dynamic collectivities compared to neutral mutations in crystal structures. On the other hand, preliminary analysis using AlphaFold3 with its basic protocol revealed that predicted mutant structures exhibit less and inconsequential dynamic differences compared to crystal structures between wild-type and mutant structures,

Conclusion: AF excels in predicting protein structures, but its present form has limitations in accurately modeling the dynamic movements that may possibly occur upon mutations, which can lead to inaccurate assessments of functional implications.

Keywords: AlphaFold, Mutations, GNM

P119
Differential Expression Profile of miRNAs Indicates that HGF Priming Ameliorates Fibrosis Related miRNAs in Bone Marrow-derived Mesenchymal Stem Cells

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Background/Aim: Bone Marrow Mesenchymal stem cells (MSCs) secrete anti-fibrotic miRNAs, which may mitigate fibrosis. Priming strategies may improve MSCs' antifibrotic behaviour. HGF is a growth factor which is used to enhance MSC regenerative potential. This study aimed to investigate HGF-priming effects on fibrosis-related miRNA expression in MSCs compared to non-primed culture conditions.

Methods: Cell viability, migration and proliferation were examined by WST, wound healing and population doubling assays, respectively. miRNA differential expression profile was obtained by using focused PCR panel (Qiagen), including 84 fibrosis-related miRNAs. More than two-fold expression level changes were determined using Geneglobe analysis tool and their common targets were evaluated using DAVID functional enrichment tool. An *in vitro* fibrosis model was set-up by inducing MSCs with 10 ng/mL TGF-β and co-cultured with HGF-primed or non-primed MSCs using a transwell system.

Results: HGF accelarated wound healing, restricted population doubling of MSCs and did not affect the cell viability. miR-32-5p (fibrotic) and miR-377-3p (anti-fibrotic) were up-regulated and miR-10a-5p (fibrotic) was down-regulated in HGF-primed MSCs. DAVID enrichment analysis has shown that common targets of these miRNAs affect TGFbeta, Wnt and Hippo pathways. *ACTA2*, *COL1A1* and *FN* expression levels in the co-cultured cells were similar with both unprimed and primed MSCs.

Conclusion: Our analysis indicated that HGF priming ameliorates expression levels of fibrosis related miRNAs in MSCs. However, the co-culture assay did not indicate a fibrosis-resolving effect of HGF primed MSCs. Further functional assays are needed with the differential expressed miRNAs to unveil the effect of HGF-primed MSCs on fibrosis.

Keywords: MSC, fibrosis, HGF, miRNA

P120
Identifying the Roles of Adipose Tissue NIK Kinase in Inflammation and Energy Metabolism

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Background/aim: The global obesity epidemic is a pressing health concern affecting diverse patient populations. Chronic inflammation caused by obesity leads to insulin resistance in patients and dysregulation of glucose metabolism is observed. Browning of the white adipose tissue can contribute to solve these issues via increasing thermogenesis. To fight these problems, the activity of the non-canonical NF-κB pathway can be modulated to attenuate inflammation related effects while inducing browning. For this purpose NF-κB inducing kinase (NIK) can be targeted.

Materials and methods: To study the role of NIK in adipose tissue inflammation and browning NIK deficient mice were euthanized and inguinal white adipose tissue is collected. Pre-adipocytes isolated from the adipose tissue and induced to differentiate into mature adipocytes upon treatment with a differentiation cocktail. Then mature adipocytes are treated with LPS, insulin and FGF21 to study adipose tissue inflammation, insulin response and browning respectively.

Results: When mature adipocytes treated with LPS in order to see inflammation responses, NIK KO cells showed reduced immune responses compared to their wild type counterparts. Second treatment done with insulin for investigating glucose mechanism responses in mature adipose cells. Upon insulin treatment NIK KO adipose cells showed increased mRNA levels for lipogenesis and glucose transport pathways which may be assessed as improved insulin sensitivity. Last treatment done with FGF21 for stimulating browning pathways. Mature NIK deficient adipocytes showed improved gene expressions for browning and thermogenic gene markers.

Conclusion: When the NIK kinase is knocked out in inguinal white adipose tissue browning, insulin signaling/glucose responses and inflammation responses are increased. Therefore, non-canonical NF-κB governed by NIK can be used as effective tool against major problems caused by obesity.

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Keywords: Obesity, Adipose tissue, NF-¿½B, Inflammation, Browning

P121
Nutrient Restriction and Lysosomal Alkalinization Remodel Apoptosis Signaling Through Lipid Oxidation, Leading to Metastatic Transformation

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Background & Aim: Limited nutrient access activates adaptive responses including apoptosis and acquisition of an invasive phenotype. We have previously shown that lysosomal alkalinization with Bafilomycin-A1 (Baf) under nutrient deficiency (ND) induced partial epithelial-to-mesenchymal transition (p-EMT) in a sub-population of Caco-2 colorectal cancer cells, which also showed enhanced pro-apoptotic signals. We have hypothesized that the decision signals for cell survival or death in response to nutrient stress might be transmitted through a rewiring of lipid metabolism.

Materials & Methods: To address this hypothesis, Caco-2 cells were incubated in an ND medium containing 10% of the nutrients found in complete culture medium and co-treated with Baf (ND+Baf). High throughput transcriptomics data generated from ND+Baf cells was processed to model how the cell decides its fate between EMT or apoptosis. The *in silico* analyses were supported by expression data, fluorescent imaging and cell viability assays to analyze the changes in EMT, apoptosis and lipid metabolism.

Results: Pathway-enrichment analysis revealed an interconnected network of cell-death mechanisms and activation of fatty-acid metabolism in ND+Baf cells. Reduced levels of lipid droplets visualized with Nile-Red staining indicated the utilization of fatty acids as energy source under ND. The analysis of fatty acid synthase (FASN) and phospho-Acetyl-CoA Carboxylase (p-ACC) levels supported the increased demand for lipids in starved cells. The same population of cells showed increased pro-apoptotic signaling as indicated by enhanced PARP cleavage and decreased viability, which was reversed with apoptosis inhibitor Q-VD-OpH. Acquired mesenchymal phenotype and increased cleaved E-cadherin levels induced with ND+Baf were also reversed when cells were treated with the apoptosis inhibitor.

Conclusion: Cancer cells can rewire their metabolism in response to environmental cues in their decision for the activation of adaptive responses. Our data shows the changes in lipid metabolism may help in the decision between cell death and survival in response to lysosomal alkalinization and nutrient restriction.

Keywords: Nutrient Restriction, Lysosomal Alkalinization, Apoptosis, EMT, Lipid Metabolism

P122
Understanding the Effect of the Inhibition of NF-kappa B Inducing Kinase (NIK) in Muscle Atrophy

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Background/Aim: Skeletal muscle atrophy is characterized by the over-catabolism of protein molecules and is linked to cancer-related cachexia. It was previously shown that non-canonical NFκB pathway activation leads to muscle atrophy. NFκB inducing kinase (NIK) is a serine/threonine-specific protein kinase in the non-canonical pathway that is maintained at low levels under basal conditions. It can be activated by members of tumor necrosis factor receptor (TNFR) superfamily, such as ectodysplasin A2 Receptor (EDA2R). NIK activation leads to the processing of p100-NFκB2 into p52-NFκB2 and the expression of muscle atrophy-related genes. Inhibiting NIK can be a potential therapeutic approach for cachectic cancer patients. SMI1 is a small molecule inhibitor that inhibits NIK-catalyzed hydrolysis of ATP to ADP, which could be used for the benefit of the patients.

Materials and Methods: Muscle atrophy is detected in RNA level by RT-qPCR and in protein level by western blotting in primary muscle cells. Cellular atrophy is visualized with immunofluorescence staining and quantified by measuring myotube diameter.

Results: Non-canonical NFκB pathway activation by NIK overexpression in primary muscle cells leads to cellular atrophy. Similar effects can be observed with the activation of EDA2R signaling by ectodysplasin A2 (EDA-A2). To study the effects of NIK inhibitor SMI1 in EDA-A2 driven activation, combined treatment with EDA-A2 recombinant protein and SMI1 was conducted in primary muscle cells. SMI1 treatment blocked NFκB pathway signaling. The combined treatment also blunted the pathway activation and inhibited the expression of muscle atrophy genes. The mobility shift of NIK protein that can be observed with EDA-A2 treatment also disappeared upon co-treatment with SMI1.

Conclusion: EDA-A2 can no longer stimulate atrophy in primary myotubes when co-treated with SMI1. SMI1 blocks NIK activity, which results in inhibition of p100 processing and muscle atrophy. Thus, administration of SMI1 poses a potential therapy for patients suffering from cancer cachexia.

Keywords: muscle atrophy, non-canonical NFκB signaling, NIK inhibition

P123
Investigation of the Metabolic Effects of VDR rs731236 and VDBP rs4588 polymorphisms in MODY Patients.

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Objectives: Fat-soluble Vitamin D functions with its receptor and vitamin-D-binding-protein and vitamin D receptor-VDR and Vitamin D Binding Protein-VDBP mutations play a role in the pathogenesis of diabetes. We aimed to investigate the gene variations of VDR and VDBP and their effects on clinical features in patients with clinically-diagnosed maturity-onset-diabetes-of-the-young (MODY).

Materials and Methods: VDR TaqI-rs731236 and VDBPStyI-rs4588 polimorphisms are analyzed by PCR-RFLP in 63 patients with MODY and 61 healthy-control subjects. Statistical analyses were performed by SPSS v.24.0.

Results: In the MODY patient group, family history, BMI, waist-circumference, fasting-blood-sugar, HbA1c and CRP ($p<0.001$), hip-circumference ($p=0.020$), TG ($p=0.008$), GGT ($p=0.010$), fT4 ($p=0.005$) and weight ($p=0.021$) levels were higher and HDL-C ($p=0.043$) and TSH levels ($p=0.020$) were lower compared to the control group.
When the effect of VDR-rs731236-(T>C) polymorphism genotypes on biochemical and clinical parameters in the MODY patient group was examined, waist-circumference was higher ($p=0.042$) while ALT ($p=0.010$), AST ($p<0.001$), GGT ($p=0.023$) and microalbuminuria ($p=0.003$) levels were lower in those carrying the normal-TT genotype compared to those carrying the mutant-C allele.
When the effect of VDBP-rs4588-(C>A) polymorphism genotypes on biochemical and clinical parameters in the MODY patient group was examined, age ($p=0.009$), GGT ($p=0.011$) and urea ($p=0.010$) were significantly higher while fT4 (0.046) and microalbuminuria ($p=0.043$) levels were lower in individuals carrying the normal-CC genotype compared to mutant-A allele carriers. GGT ($p=0.004$ and $p=0.008$, respectively), urea ($p=0.027$ and $p=0.031$, respectively) and creatinine ($p=0.036$ and $p=0.028$, respectively) levels were found highly correlated with CC genotype compared to CA and AA genotypes.

Conclusions: Our findings show that the VDR rs731236 T>C (TaqI) mutant CC genotype and the VDBP rs4588 C>A (StyI) normal CC genotype were effective on parameters related to kidney and liver functions. Therefore, further studies with larger sample sizes are required for stronger findings.

Keywords: MODY, VDR, VDBP, rs731236, rs4588

P124
Investigation of m6A RNA Methylation Effects in Triple Negative Breast Cancer Cells

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Background:
Epitranscriptomics explores more than 170 RNA modifications including N^6-methyladenosine (m6A) that play critical roles in various diseases such as cancer. As the most prevalent form of methylation, m6A has garnered substantial attention for its potential applications in cancer diagnosis and treatment. However, the impact of m6A RNA methylation in HCC1143, triple-negative breast cancer, remains elusive. This study aims to evaluate phenotypic effects of m6A RNA methylation in HCC1143 triple-negative breast cancer cell line after knock-down of METTL3 that has the catalytic activity to add m6A on mRNA.

Methods: The knockdown of METTL3 was performed by using siRNAs. After the knock-down of METTL3, the phenotypic impact of METTL3 silencing was analysed. The viability assay was carried out by WST8 Colorimetric Assay. Then, apoptosis rate of cells was measured by Annexin-V-FITC and 7AAD-PerCP dyes while cell cycle analysis was performed by propidium iodide (PI) staining in flow cytometry. Lastly, RNA sequencing was carried out after METTL3 knock-down by comparing with off-target siRNA.

Results: The maximum m6A reduction was detected at 72h after METTL3 transfection. The cell viability decreased as 40.1% in METTL3 silencing. To determine the reason for the decrease in viability, apoptosis and cell cycle analyses were performed. Although there was no change in the apoptotic rate, 34.27% G2/M phase arrest was captured upon METTL3 knock-down in HCC1143 cells. 585 differentially expressed genes (DEGs), as determined by RNA sequencing, were used to perform Gene Ontology Enrichment Analysis, which showed that the cell migration related pathways were highly affected upon METTL3 knock-down.

Conclusion: The silencing of METTL3 results in 40% of m6A reduction and 585 DEGs, mostly related to cell migration and motility pathways. The candidate genes associated with cell migration and G2/M phase were identified to gain further insight into the function of METTL3 in HCC1143 cells.

Keywords: N6-methyladenosine, Viability, G2M phase arrest, Cell migration, HCC1143

P125
Identification of PBP3 Inhibitors by Covalent Docking-Based Virtual Screening

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Background/Aim: Antimicrobial resistance to medications is recognized as a worldwide public health issue. Penicillin Binding Proteins (PBPs) are found in all bacteria and are important targets in anti-biotherapy, particularly for commonly used β-lactam antibiotics.
Staphylococcus aureus
is a common gram-positive pathogen and is difficult to treat clinically, especially a methicillin-resistant form.
S. aureus
PBP3 serves an important function in accelerating the cross-linking of cell wall peptidoglycan during cell division. PBP3 was thus selected as a promising therapeutic target in order to find effective inhibitors that may be used as antibacterial medication options. In the light of this information, it is aimed to computationally determine PBP3 covalent inhibitors in drug molecules (FDA-approved, experimental, and research) in the Drugbank database.

Materials and Methods: All molecules (~10767) in the Drugbank database were downloaded and 2D structures were converted into 3D structures by preparing them with the LigPrep module. PBP3 crystal structure was prepared using the Protein Preparation Wizard. Virtual screening was performed with the Glide covalent docking algorithm and then the clustering with the Tanimoto similarity matrix resulting from ligand-based similarities. Hit compound were found by evaluating clustering and covalent docking results. Next, Desmond was used to analyze molecular dynamics (200 ns) of the hit and Cefotaxim (Reference)-PBP3 complexes.

Results: As a result of the virtual screening, docking scores were examined, interactions in the clusters were evaluated, and Terbogrel was selected as the hit drug molecule. It was determined that Terbogrel formed a covalent bond with Ser392 and found to be in the binding cavity during the period of simulation to be stable and to have at lower RMSD (Cα and (lig) fit on prot) value compared to the reference compound.

Conclusion: In this study, it was found that Terbogrel could be a potential novel PBP3 covalent inhibitor for the treatment of bacterial infections.

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Keywords: PBP3, Covalent Doking, Molecular Simulation, Virtual Screening

P126
Investigation of the regenerative potential of proteoglycan 4a in the brain in zebrafish model of traumatic brain injury and demyelination

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Brain regeneration is crucial to preserve normal brain function after an injury. Although the human brain has a very limited ability to repair injuries and regenerate neurons, the vertebrate model zebrafish can regenerate almost all its tissues and organs, including the central nervous system (CNS). This capability makes zebrafish a highly convenient *in vivo* model to investigate the molecular mechanisms of regeneration in response to brain injury. Proteoglycans regulate CNS repair and regeneration through interacting with cell adhesion molecules, receptors, and neuroregulatory proteins. Proteoglycan 4a (Prg4a) plays significant roles in the inhibition of neuroinflammation and restore blood-brain barrier function due to traumatic brain injury (TBI). Yet, the dynamic expression profile and functional role of Prg4a in the regeneration after brain injury remain unknown. Therefore, our aim is to unravel the functional roles of Prg4a during regeneration due to TBI and non-traumatic brain injury (NTBI) comparatively. In our study, we analyze the role of Prg4a in regeneration after TBI and NTBI in zebrafish at the cellular and molecular levels using loss-of-function and gain-of-function approaches and investigate the Prg4a-related cellular pathways and proteins through proteomic methods. Our previous studies in which we analyzed differentially regulated genes during zebrafish brain regeneration, showed a dramatic increase in Prg4a expression compared to the uninjured control during regeneration following TBI. Considering that, we confirmed the upregulation of Prg4a expression after brain injury at both the RNA and protein levels. This study will be the first to reveal the roles of Prg4a during regeneration in TBI and NTBI models and elucidate the development of new treatment options against brain injuries.

Keywords: zebrafish, neuronal regeneration, proteoglycan 4a

P127
Association of ASIC1 Gene Variations with Coronary Artery Disease Risk

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Background/Aim: Coronary artery disease (CAD) is the leading cause of death worldwide. Genetic factors play a significant role in CAD, with many loci containing genes linked to the disease. Acid-sensing ion channels (ASICs) are proton-gated cation channels found in various tissues and are activated by low extracellular pH. There are seven known ASIC members: ASIC1a, ASIC1b, ASIC1b2, ASIC2a, ASIC2b, ASIC3, and ASIC4. The acidification of intimal fluid is believed to be important in the development of CAD. This study investigates whether variations in the ASIC1 gene region could be risk factors for CAD.

Materials and Methods: The study involved 80 individuals diagnosed with CAD and 81 healthy individuals, all of whom underwent coronary angiography at Mersin University Faculty of Medicine Hospital Cardiology outpatient clinic. Genetic variations were identified using the Sanger sequencing method.

Results: DNA sequence analysis revealed two variations in the ASIC1 gene: rs706793 and rs706792. Genotype distributions in all groups were consistent with Hardy-Weinberg Equilibrium according to $\chi 2$ goodness-of-fit tests. Odds Ratio analysis suggested a potential link between these variations and CAD. The rs706793 variation did not pose a risk for CAD. However, the rs706792 polymorphism was 12.2% more frequent in the patient group compared to the control group ($p<0.05$), indicating its possible role as a genetic risk factor for CAD.

Conclusions:
Larger studies are needed to confirm these findings across different genetic populations.

This study was supported by Mersin University Scientific Research Projects Coordination Unit (Project Number: (2021-1-TP3-4171).

Keywords: Coronary Artery Disease, Atherosclerosis, Acid-Sensing Ion Channel 1, rs706793, rs706792

P128
The Regulation of SMAD-Independent Pathways of TGF-β Signaling in Hypoxia in the Prostate Cancer Cell Model (PC-3)

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TGF-β (Transforming Growth Factor-β) family functions in many processes such as cell proliferation, cell growth, cell differentiation, and apoptosis. TGF-β signal transduction pathway carries out this process in SMAD-dependent and SMAD-independent. It is a tumor suppressor for early-stage tumors and a potent growth inhibitor of cells of epithelial origin, but in advanced cancers, it promotes tumor growth and progression by inducing epithelial-to-mesenchymal transition (EMT) and subsequent tumor invasion and metastasis. It provides activation of JNK (c-Jun N-terminal kinase) and p38 MAPK signaling pathways as a result of ligand binding to TGF-β receptors. TGF-β and BMP have also been shown to activate Akt through PI3K(Fosfatidilinositol-3-Kinaz), and studies have also shown that it is activated by SMAD-independent or SMAD-dependent mechanisms. Many studies have also shown that (PI3K)/AKT, SAPK(stress-activated protein kinases)/JNK, and p38 pathways are activated independently of SMAD in the TGF-β signaling pathway. In our study, the signaling pathways activated by TGF-β in the chemical hypoxia model were investigated at the protein level.

PC-3 cells were grown in DMEM F12 Mix containing 10% FCS medium. $CoCl_2$ (150 µM), a hypoxic agent, and the cytokine TGF-β1 (20ng/ml) were applied to the cells for hypoxia and TGF-β stimulation. PI3 Kinase p85 (Phosphatidylinositol 3-kinase), SAPK/JNK, P38 MAPK, and β-Actin primary antibodies were used for protein analyses using western blot. Bands were evaluated in Image J and normalized with β-actin. These experiments were performed independently of each other in at least 2 replicates.

In this study, where the SMAD-independent pathways of the TGF-β signaling pathway were studied, it was seen that TGF-β or $CoCl_2$ induces the PI3K, SAPK/JNK, and p38/MAPK pathways. It was noteworthy that this situation was seen especially in early time zones. Under hypoxic conditions, TGF-β maintained this effect only on the PI3K pathway in PC-3 cells. This expression was especially reduced in hypoxic TGF-β in the SAPK/JNK and p38/MAPK pathways.

Keywords: Hypoxia, Prostate cancer, SMAD Pathways, TGF-b

P129
Determination of the Effect of EF24 on Cisplatin-induced Cell Death Response in Triple Negative Breast Cancer Cells

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Background/Aim: Triple-negative breast cancer is a highly aggressive subtype of breast cancer that lacks estrogen and progesterone hormone receptors, and human epidermal growth factor receptor 2. Chemotherapy is still not promising for the treatment of TNBC. Therefore, new pharmaceutical research has focused on identifying and inhibiting molecular pathways that play a role in the proliferation and development of TNBC.

Materials and Methods: The effects of the EF24 and cisplatin on MDA-MB-231 and MDA-MB-436 cells were evaluated in terms of cell viability, cell migration, and cleaved caspase 9 levels. Varying concentrations of EF24 (1-32 μM) and cisplatin (5-50 μM) were applied to MDA-MB-231 and MDA-MB-436 cell lines, both individually and in combination. The effect of combined applications of EF24 and cisplatin at selected concentrations on the migration ability of cells was determined using a wound healing assay. Active caspase-9 protein levels were evaluated by Western blot.

Results: In MDA-MB-231 cells, the cell viability rate in single-agent treatments of EF24 and cisplatin was found to be higher compared to MDA-MB-436 cells at 24 and 48 hours. However, it was determined that the cell viability rate in combined treatments at 24 and 48 hours was lower in MDA-MB-231 cells. The rate of wound closure was calculated. Compared to MDA-MB-231 cells, it was determined that there was an increase in the migration rate of MDA-MB-436 cells when treated with EF24 and cisplatin, both individually and in combination at 24 and 48 hours. In both cell lines, no difference was detected in the levels of active caspase-9 in single and combined treatments of the agents at the selected concentrations ($p>0.05$).

Conclusion: To determine the type of cell death observed in both cell lines, our future studies aim to investigate whether the extrinsic pathway of apoptosis is activated and to examine the presence of autophagy markers.

Keywords: triple negative breast cancer cell lines, EF24, cisplatin

P130
The Biophysical Landscape of Reconstituted Immune Synapses

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Background/Aim: The immune synapse is a spatiotemporally highly organized cell-cell contact between immune cells and their target cells, in which signalling events are tightly controlled. The nature and dynamics of these protein-protein interactions at the immune synapse are strongly influenced by the plasma membrane biophysical properties. Dissecting the influence of specific membrane properties on individual protein-protein interactions or vice versa is not trivial. Therefore, we aim to generate a synthetic immune synapse allowing detailed investigation of biophysical properties alongside protein-protein interactions, to gain a comprehensive understanding of molecular mechanisms at the immune synapse.

Material and Methods The immune synapse was reconstituted using model membranes (giant unilamellar vesicles, GUVs) decorated with proteins of interest alone or together with live cells. The formation of the contact as well as enrichment or exclusion of proteins at the contact were quantified by confocal imaging. Using environment-sensitive fluorescent probes and spectral imaging, we examined the biophysical properties at the synthetic immune synapse. Diffusion dynamics within the contact were explored with line scanning fluorescence cross correlation spectroscopy.

Results: At both the purely artificial (GUVs only) and semi-artificial (GUV & Jurkat T cell) contacts, adhesion and immune ligand-receptor pairs such as CD2 & CD58 or pMHC & TCR, among others, are strongly enriched. The phosphatase CD45 and heavily glycosylated proteins CD43 and MUC1 are excluded from the contact. Small membrane fluidity changes were observed at the contact compared to non-contact membranes.

Conclusion: In summary, we could create a straightforward and widely applicable cell-cell contact reconstitution model, which enables investigation of contact formation, protein enrichment or exclusion, diffusion dynamics as well as biophysical properties at the immune synapse.

Keywords: membrane properties, immunology, immune signaling, immune synapse, model membrane

P131
Effect Of Hesperidin Loaded pH And Temperature Sensitive Smart Nanoparticles On Breast Cancer

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Background/Aim: Breast cancer is the most common type of cancer encountered by women worldwide. Although hesperidin is a promising anticancer agent, its poor water solubility and bioavailability limit its use as a therapeutic agent. In this study, it was aimed to improve bioavailability and increase therapeutic efficacy against breast cancer by experimentally preparing pH and temperature sensitive smart polymeric nanoparticles carrying hesperidin.

Materials and Methods: 2-hydroxyethyl methacrylate was used to synthesize temperature-sensitive nanoparticles, and vinyl imidazole was used for pH-sensitive nanoparticles. Particle size and size distribution of hesperidin-loaded nanoparticles were determined by dynamic light scattering analysis. Scanning electron microscopy was used to determine morphological characterization. Additionally, the effects of hesperidin-loaded smart nanoparticles on cell proliferation, apoptosis and cell cycle in breast cancer were also examined. MTT assay was used to evaluate the cytotoxic effects of smart nanoparticles on MCF-7 cells at different concentrations for 24 h.

Results: It was found that MCF-7 cell proliferation in experimental groups and free hesperidin group decreased statistically significantly compared to the control group, depending on the dose ($p<0.05$). The apoptotic effect was examined with the Annexin V-PI Staining Method in flow cytometry. The increase in the rate of early and late apoptosis cells in the experimental groups was found to be statistically significant compared to the control group. When the effect on the cell cycle was evaluated, the rate of cells in S phase decreased compared to the control group.

Conclusions: As a result, it was determined that hesperidin-loaded pH and temperature sensitive smart nanoparticles showed cytotoxic, apoptotic and antiproliferative effects on the MCF-7 cell line. According to the data obtained through this study, new studies will be planned on active substances with bioavailability problems or cancer drugs that cannot achieve the expected effect in their free form.

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Keywords: MCF-7, breast cancer, nanoparticle, hesperidin

P132
Effect of Imatinib and Calcitriol Combination on Telomerase Activity in K562 Chronic Myeloid Leukemia Cells by Cost-Effective Manual TRAP qPCR Method

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Today, the standard treatment for CML is imatinib, which is revolutionary in the treatment of CML; however, despite the great clinical progress it has provided, imatinib resistance observed in the clinic can pose a problem in terms of treatment. In order to eliminate this problem, different drugs and different
combinations are tried as imatinib replacements.
In our study, we aimed to develop a more effective method for CML treatment and to pave the way for translational studies that can provide a more accessible treatment option for cancer patients by combining calcitriol, the active form of vitamin D, which is increasingly deficient in individuals in the world's populations, is convenient in terms of accessibility and cost, and is known to have an effect on telomerase activity based on literature, with imatinib.
For this purpose, imatinib, calcitriol and the combination were applied to K562 and HEK293 cells. After the cell lysis, the protein and DNA concentration of K562 and HEK293 lysates were measured using NanoDrop. Telomerase activity determination was performed with the cost-effective manual TRAP qPCR method. The HEK293 cell line, which has high telomerase activity, was used as the control group. Statistical analyses were performed with the Kruskal Wallis non-parametric ANOVA test method.
We have found that; imatinib, calcitriol, and combination applied K562 cells had higher telomerase activity than untreated cells, whereas HEK293 cells had lower telomerase activity.
Our results have demonstrated that imatinib, calcitriol, and their combination did not improve telomerase
inactivation in CML cell line K562, even though they all were successful in the inactivation in embryonic cell line HEK293. Although the efficacy of the combination of imatinib and calcitriol has been examined in other hematological cancer types including CML, no research has been conducted
on this combination before. According to the data we have obtained, the potential of a novel approach that complement the current treatment is investigated.

Keywords: chronic myeloid leukemia, K562, telomerase, calcitriol, imatinib

P133
Investigation Of MAN2A1 Gene Expression In Schizophrenia Patients In Association With Neuroplasticity

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Background/Aim: Schizophrenia is a heterogeneous psychotic illness that is caused by various genetic and environmental factors. Neuroplasticity is the brain's ability to change and adapt in response to internal and external stimuli by changing, reorganizing or growing neural networks. In schizophrenia, alterations in neuroplasticity can result in molecular changes in specific neural networks or modulation of neurotransmitter receptor function.
MAN2A1 encodes a glycosyl hydrolase that catalyses the last hydrolytic step in the asparagine-linked oligosaccharide maturation pathway in the Golgi apparatus and has recently been linked to intelligence and general cognitive ability. *MAN2A1* is known to be involved in schizophrenia. Aim of this study, therefore was to investigate *MAN2A1* gene expression in schizophrenia patients in association with neuroplasticity and relation to *CREB1* expression, an important neuroplasticity gene.

Materials and Methods: Peripheral blood samples, taken from 47 study group subjects with schizophrenia and 17 healthy subjects as control, were analysed for *MAN2A1* and *CREB1* gene expressions by a real-time qRT-PCR method, following total RNA isolation and cDNA synthesis. The relative fold gene expressions of the samples were calculated with the $2^{-\Delta\Delta Ct}$ method. Demographic data of the study group subjects were also analysed by using GraphPad Prism.

Results: *CREB1* gene expression was found to be decreased in the study group subjects when compared to the control group subjects ($p<0.05$); while, *MAN2A1* gene expression was found to be similar between both groups. No significant association was found between the demographic data of the study group subjects and *MAN2A1* / *CREB1* gene expressions.

Conclusion: In this study, no changes in *MAN2A1* gene expression could be observed in schizophrenia patients. It will be of interest to further investigate its possible relationship with neuroplasticity with more subjects and advanced techniques.

This work was supported by Ege University Scientific Research Projects Coordination (TSYLT-2023-30761 to B.K.).

Keywords: schizophrenia, MAN2A1, CREB1, neuroplasticity, gene expression

P134
Establishment of Irinotecan Resistant LIM 1215 Cell Lines, Identification of Gene Expression Changes and Discovering Potential Second Line Drugs

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Introduction: Irinotecan, a topoisomerase I inhibitor, is one of the widely used drugs in clinic for the treatment of Colorectal cancer (CRC), which is the third most common type of cancer. Unfortunately, CRC patients frequently develop resistance to irinotecan treatment. Our study aims to investigate the underlying mechanisms of irinotecan resistance in LIM 1215 cell line, where acquired irinotecan resistance was induced
in vitro, as well as to identify potential second-line therapeutics that can revert the drug resistance.

Materials & Methods: LIM 1215 cells were exposed to IC 50 dose of Irinotecan for over a year to develop its resistant derivatives in 2 parallel biological replicates. Following this, the irinotecan-resistant cells were divided into two groups: one group continued to be exposed to irinotecan, while the other group was not exposed to irinotecan. To identify gene expression changes in these two experimental groups, bulk RNA sequencing was performed. Furthermore, a drug screening assay was conducted using a library of 50 drugs to identify potential agents that could sensitize the two experimental groups.

Results: The irinotecan-resistant LIM 1215 cell lines showed morphological changes with elongated structures, resembling an EMT phenotype, compared to the parental cell line. However, these morphological changes reversed once the drug was removed. RNA-seq analysis demonstrated gene expression differences between the parental cell line, the drug-resistant cell line and the cell line where the drug was removed. Both morphological and RNA-seq analyses showed differences between the resistant and drug-sensitive cell lines, as well as changes after drug removal from the resistant cell line. The drug screening assay identified potential therapeutic agents to overcome irinotecan resistance.

Conclusion: Irinotecan resistance was established in the LIM 1215 cell line. Transcriptomic changes were analyzed in groups continuing irinotecan treatment and after drug removal. Secondary drug sensitivities were assessed for both groups to improve treatment strategies for CRC patients with irinotecan resistance.

Keywords: colorectal cancer, drug resistance, drug screening

P135
Detailed Bioinformatics Analysis of PUF60 and Its Role in Cancer Development

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Background/Aim: Alternative splicing (α-EJ) occurs in ~95% of human genes and can play a critical role in disease development; e.g. by directly affecting cell proliferation, invasion, migration and apoptosis of cancer cells. We recently have identified PU60, a protein with important roles in pre-mRNA splicing and transcriptional regulation, as candidate gene for inducing cisplatin resistance in non-small cell lung cancer CR-Calu1 cells by a genome-wide CRISPR-Cas9-based genetic screening method. The aim of this study, therefore was to further analyse PUF60 and determine its additional roles in cancer biology by means of bioinformatics.

Materials and Methods: Analyses were carried out with various bioinformatics tools and databases such as UniProt, cBioportal, TCGA, NCBI, PDB, BLAST, CLUSTAL Omega, String and Gepia. Cancer-related mutations and functions were analysed. Homology modelling studies were performed to demonstrate high degree of conservation. Protein-protein interaction networks were used to provide supporting evidence for PUF60's roles in cellular processes.

Results: Abnormal expression and mutations of PUF60 have been revealed for various cancer types. PUF60 overexpression has been linked to increased viability and colony formation of cancer cells. Some PUF60 isoforms were found to be involved in tumor progression by conferring increased MYC expression and greater resistance to apoptosis. Homology modelling studies revealed its high degree of conservation.

Conclusion: Bioinformatics analyses of PUF60 highlighted its importance in cancer biology. Further functional studies, like determining its interactions with other proteins and their effects on cellular processes, will reveal and support its potential as novel therapeutic target in cancer treatment.

Keywords: Bioinformatics, Cancer, PUF60, Mutations

P136
Production of High-Fidelity Pfu DNA Polymerase

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DNA-polymerases are important tools in molecular biology, particularly in polymerase chain reactions (PCR) to amplify genes of interest to facilitate genetic engineering.
Pfu DNA-polymerase is a high-fidelity product that has better thermostability compared to other polymerases. It also possesses a 3' to 5' proofreading ability that enables correction of errors in the 3' ends. This feature ensures error-free PCR products.
We recently generated a pET-16b bacterial expression plasmid which contains the Pfu DNA-polymerase gene. Our aim was to verify the presence of the polymerase gene and purify the enzyme at the protein level to aid studies.
To determine the plasmid concentration, several dilutions were prepared and transformed into DH5α cells. Later, plasmids were isolated by the Miniprep method and diagnostic digestion was performed with BsaI and StuI enzymes which verified the presence of the Pfu sequence. For protein production, plasmids were transformed into BL21 AI cell line and cells were induced with arabinose and IPTG for protein expression.
Cells were sonicated and heated up to 80 degrees to precipitate irrelevant bacterial proteins to enrich the polymerase enzyme. This enzyme was purified using Immobilized metal affinity chromatography (IMAC), targeting the His-tag at the N-terminus of the Pfu protein. To remove imidazole, which could interfere with PCR reaction, an additional size exclusion chromatography step was performed.
In conclusion, we were able to isolate and purify Pfu DNA-polymerase. We further conducted several PCR reactions using this enzyme to confirm its functionality and to compare its activity with other DNA-polymerases. In-house expressed and purified Pfu demonstrated adequate activity to conduct our further experiments with our own purified enzyme.
In conclusion, to utilize Pfu as a first line enzyme in our studies, we have optimized the Pfu production procedure. We also require determining the optimal storage conditions for Pfu, so that our further studies will be focusing on developing formulation buffers for both short-term/long-term storage.

Keywords: DNA Polymerase, PCR, protein purification

P137
VEGF-A upregulates ADAMTS-8 expression in SW480 cells

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Background/Aim:
A disintegrin and metalloprotease with thrombospondin motifs (ADAMTSs) are a family of extracellular matrix metalloproteinases having critical functions in a variety of physiological processes including extracellular matrix degradation, hemostasis, organogenesis, and angiogenesis. Abnormal expression of some ADAMTS members has been found to be related to tumor development and progression. ADAMTS-8 is an antiangiogenic member of the ADAMTS family. It has been demonstrated that ADAMTS-8 can inhibit vascular endothelial growth factor (VEGF)-mediated angiogenesis in endothelial cells. Growing evidence indicates that epigenetic silencing of ADAMTS-8 is important in tumorigenesis. The tumor suppressor function of the ADAMTS-8 has been demonstrated in colorectal cancer (CRC). VEGF has been known as a strong promoter of tumor-related angiogenesis. Expression of the VEGF in the early stage of CRC is correlated with microvessel formation and poor outcomes. Some ADAMTS members, such as ADAMTS-1 have been shown to inhibit the VEGF expression and further cancer cell proliferation.
However, the effect of the VEGF on the ADAMTS-8, expression remains unknown. In the current study, we aimed to elucidate the effect of the VEGF on ADAMTS-8 transcriptional regulation.

Material and Methods: SW480 cells were chosen as the CRC cell model. Cells were stimulated with 20ng/ml of VEGF-A. The effect of the VEGF-A on ADAMTS-8 mRNA and protein expressions was determined by qRT-PCR and western-blot in different incubation times.

Results: It has been determined that ADAMTS-8 significantly induced ADAMTS-8 mRNA and protein expressions in SW480 cells

Conclusion: These findings indicated that ADAMTS-8 expression is able to regulate by VEGF-A in SW480 cells.
This study is supported by the Health Institutes of Türkiye (TÜSEB) (Project Number: 33168).
Keywords: ADAMTS-8, VEGF-A, SW480, Colon Cancer

P139
The Role of ZBTB Transcription Factors in Immunoglobulin Class Switch Recombination

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The human genome encodes 49 different ZBTB family proteins which function in cell proliferation, DNA damage responses, and gene repression. Their N-termini contains BTB domains that are responsible for protein-protein interactions and their C-termini contains zinc finger motifs that bind nucleic acids. These proteins form homodimers. Furthermore, PATZ1 and PATZ2 (also named as ZBTB24) were observed to form heterodimers. ZBTB24 was discovered to bind poly-adenosine ribose (PAR) chains synthesized by the PARP-1 enzyme as a DNA damage response The aim of this study is to discover roles of other ZBTB proteins in the double-stranded break response. Mutant ZBTB24 causes immunodeficiency, chromosomal instability, facial anomalies 2 (ICF2) syndrome, a rare disease affecting Turkish families.
We will investigate the function of 10 other ZBTB family proteins to explore their effects on class switch recombination (CSR). We will use the CH12 mouse cell line that particularly undergoes CSR such that IgM is replaced by IgA. Using CRISPR/Cas9, we will generate knock-out cells regarding ZBTB20, ZBTB22, ZBTB24, ZBTB28 (BCL6B), ZBTB32, ZBTB33(Kaiso), BCL6, PATZ1, LRF and MIZ1. These factors were selected as they are known to be expressed in the germinal center where CSR happens during B lymphocyte development. The gene targeting strategies will be described in detail.

Keywords: ZBTB, Class Switch Recombination, ZBTB24, Dimerization, ICF2 Syndrome, CRISPR-Cas9

P140
Anti-metastatic Effects of Therapeutic Antibody Trastuzumab and Caffeic Acid Phenethyl Ester (CAPE) on Breast Cancer Cell Line MDA-MB-231

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Background/Aim: Breast Cancer is a disease in which the cells lining the mammary glands and milk ducts proliferate uncontrollably and spread to the surrounding tissues. Trastuzumab is a monoclonal antibody used in the treatment of breast cancer. However, the occurrence of side effects due to drugs seriously limits their use. Researchers have focused on determining the biological properties of natural products in line with the need to reduce these side effects and develop treatment support products and new drugs. Although hundreds of phenolic substances have been identified in the structure of propolis, a natural honeybee product, CAPE has been expressed as the most effective compound in scientific studies in terms of its biological activity. In this study, it was aimed to increase the effect of Trastuzumab with CAPE in triple negative breast cancer cell line MDA-MB-231 cells.

Materials and Methods**:** Cytotoxic doses of Trastuzumab and CAPE were determined by MTT test on MDA-MB-231 cells. The effects of Trastuzumab and CAPE on cell migration were evaluated with the wound healing assay. Wound healing images were analyzed with image J. In addition, VEGF, Snail1 and Snail2 (Slug) gene expression levels of the treated cells were amplified with Sybr Green-based primers by Real Time RT-PCR (qPCR) method and determined according to the 2-ΔΔCt formula using the 18S internal control gene primer.

Results**:** IC50 values of Trastuzumab and CAPE on MDA-MB-231 cells were determined as 11.17 µg/mL and 10.84 µM, respectively. Compared to the control group, Trastuzumab and CAPE administered alone and together prevented wound closure. Confirming this data, the expressions of VEGF, Snail1 and Snail2, which are migratory and metastatic genes, decreased.

Conclusion: It is thought that CAPE, an apitherapeutic compound, will contribute to the development of new drugs or treatment support products.

Keywords: Caffeic Acid Phenethyl Ester, Trastuzumab, MDA-MB-231 cell line, Metastasis

P141
Comparison of Expression of KLF-8 Gene in Human Endothelial Cells Under Hypoxic and Normoxic Conditions

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Background/Aim: The KLF (Kruppel-like factor) genes consists of transcription factors that regulate the expression of genes that play important roles in fundamental biological processes such as growth, development, proliferation, differentiation and apoptosis. There are 17 members of this genes and many of them have been associated with various diseases including cancer. However, there are no studies on the expression of this gene in HUVEC (Human Umbilical Vein Endothelial) cells in the literature. Based on this deficiency, the expression of KLF8 was determined at the mRNA level under hypoxic and normoxic conditions using qRT-PCR strategy.

Materials/Methods: KLF8 gene expression differences under normoxia (normal oxygen) and hypoxia (low oxygen) conditions were examined in HUVEC cells, which are vascular endothelial cells. For this purpose, HUVEC cells were cultured in DMEM medium containing 10% FCS at 37°C and 5% CO_2 incubator conditions. After 24h, the hypoxia group cells were created with 150 µM $CoCl_2$, a chemical mimic of hypoxia. Total RNA was isolated from cells in hypoxia and normoxia groups at different time points. cDNAs were analyzed by Real-Time PCR using primers for KLF-8 gene and primers for human beta microglobulin gene as internal control. Results were analyzed by Livak method and graphed using GraphPad program.

Results: KLF-8 expression was well expressed in human endothelial cells. It was found to be significantly decreased in hypoxic conditions compared to normoxic conditions at both 24 and 48 hours.

Conclusion: Determination of KLF8 gene expression in hypoxic and normoxic conditions at the basic molecular level will guide future mechanistic studies.

Keywords: KLF-8, Hypoxia, Normoxia, HUVEC

P142
VEGF upregulates ADAMTS-1 protein expression in Hep3B cells

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Background/Aim:
ADAMTS-1 is the first member of the multifunctional ADAMTS protein family, known for its anti-angiogenic and aggrecanase activities. It is deregulated in many pathophysiological conditions, with studies reporting changes in its mRNA and protein expression associated with tumor progression in various cancers. Consequently, ADAMTS-1 is considered a potential tumor suppressor gene. VEGF, a pro-inflammatory cytokine that promotes tumor growth by inhibiting cell-mediated mechanisms that destroy tumors, is suggested as a potential tumor marker in the metastasis of cancers such as hepatocellular carcinoma (HCC), the most common type of liver cancer. A recent study examined ADAMTS-1 protein levels in Hep3B cells under normal and hypoxic conditions using immunofluorescence, finding an increase in both mRNA and protein levels under both conditions. However, these results were not confirmed by Western Blot analysis. In this study, we aim to investigate the effect of VEGF, which plays a crucial role in HCC, on the translational regulation of ADAMTS-1 using Western Blot analysis.

Materials and Methods: The effect of VEGF application on Hep3B cells, a liver cancer model, on the protein level of the ADAMTS-1 gene was determined by the Western Blot method.

Results: According to the data obtained after 1, 3, 6 and 24 hours of incubation; It was determined that VEGF application upregulated the ADAMTS-1 gene at the translational level compared to the control group in all time zones, and the highest increase occurred in the 1st time zone.

Conclusion: In line with the results obtained, the positive effect of VEGF cytokine application on the ADAMTS-1 protein level in the liver cancer Hep3B cell line was confirmed by Western Blot analysis.

This study was supported by TUBITAK 2209-A (Project Number:1919B012325744).

Keywords: ADAMTS-1, VEGF, Liver Cancer, Hep3B

P143
The Effect of Interleukin-6 Cytokine on ADAMTS-1 mRNA Level in Colon Cancer Cells

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Background/Aim: ADAMTS (A disintegrin and metalloprotease with thrombospondin motifs) is an extracellular protease family found in both mammals and invertebrates. It plays a crucial role in tissue organization, coagulation, inflammation, arthritis, angiogenesis, and cell migration. The first member of this family, ADAMTS-1, inhibits angiogenesis by increasing Vascular Endothelial Growth Factor (VEGF) and impeding vascularization by stimulating Fibroblast Growth Factor 2 (FGF2), thereby categorizing it as anti-angiogenic. Research shows that ADAMTS-1 expression is disrupted in various cancers, particularly in colon cancer cells where it is downregulated, suggesting a potential role as a tumor suppressor gene. Interleukin-6 (IL-6), a pro-inflammatory cytokine, promotes tumor growth by inhibiting the cell-mediated mechanisms that identify and eliminate tumors. IL-6 enhances colony formation in colon cancer cells, while blocking IL-6 in vivo suppresses colon cancer progression. Despite extensive research on ADAMTS-1 mRNA expression and its role in cancer, there is no study on the effect of IL-6 cytokine on ADAMTS-1 mRNA levels in SW480 cells. This research aims to determine the impact of IL-6 on ADAMTS-1 mRNA levels in SW480 cells using Real-Time PCR analysis.

Material and Methods: The SW480 cells were selected as a colon cancer cell model. The cells were stimulated with 20 ng/ml IL-6 cytokine. The effect of IL-6 on ADAMTS-1 mRNA levels was determined by Real-Time PCR analysis at different incubation times.

Result: Results from incubation periods of 3, 6, 24, and 48 hours indicated that IL-6 application upregulates the ADAMTS-1 gene at the transcriptional level across all time intervals, with the highest increase observed at 48 hours.

Conclusion: These findings indicated that ADAMTS-1 expression can be regulated by IL-6 in SW480 cells.

This study was supported by TUBITAK (Project Number:1919B012220942)

Keywords: Colon Cancer, ADAMTS-1, IL-6, SW480

P144
SP1 is Involved in Metastasis and Cell Proliferation of Hepatocellular Cancer Cells

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Background/Aim: SP1 is a significant transcription factor that regulates various cellular processes including cell differentiation, growth, apoptosis, immune responses, DNA damage response, and chromatin remodeling. SP1 has implications in the progression of different cancers. This study aims to perform the cellular and molecular characterization of SP1 transcription factor silenced in hepatocellular carcinoma.

Materials/Methods: To achieve this, lentiviral-based shRNA strategy will be employed to reduce SP1 expression in cells. Confirmation of silencing will be conducted via qRT-PCR. Cellular characterization will include colony formation assays and scratch tests. Analysis of metastatic genes will also be determined by qRT-PCR.

Results: Following bioinformatics studies of the SP1 gene, double-starnded oligos were cloned into the pLKO.1 vector. The cloned pLKO-SP1 and appropriate packaging vectors, pMD2.G, psPAX2 were transfected into HEK-293T cells to produce lentiviral fluid. Lentiviral fluid obtained in varying amounts was subsequently used to transduce Hep3B cells. Single colonies were selected through puromycin antibiotic applications. The colony demonstrating effective silencing was expanded to establish a population of silenced cells. RNA isolation, cDNA synthesis, and validation of silencing at the mRNA level were performed using qRT-PCR, revealing a 99% decrease in SP1 gene expression in Hep3B cells compared to the scrambled (SCR) group. The impact of silenced SP1 gene on Hep3B cell proliferation was assessed using MTT assay, invasion capacity using wound healing assays, and colony formation ability through colony formation assays. Results indicated a 50% reduction in cell proliferation compared to the control group, significantly reduced wound healing capacity, and a 2-fold decrease in colony formation ability. Furthermore, mRNA levels of metastasis-related genes such as e-cadherin, vimentin, and slug genes were determined in SP1 silenced cells.

Conclusion: The permanent silencing of the transcription factor Sp1 in Hep3B cells has enabled elucidation of its anti-proliferative effects in liver cancer cells, determination of antimetastatic mechanisms, and characterization of the phenotypic effects of the SP1 gene.

Keywords: SP1, Gene Silence, HCC, Hep3B

P146
FGF-induced downregulation of ADAMTS-8 mRNA in Saos-2 cells

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Background/Aim: A Disintegrin and metalloprotease with thrombospondin motifs (ADAMTSs) are extracellular matrix metalloproteinases. They are mainly cleavages of extracellular matrix components. They have critical functions in both normal and pathological circumstances. ADAMTS members is related to tumorigenesis. ADAMTS-8 is an antiangiogenic member of the ADAMTS family. It also has tumor suppressor functions in some cancer types. Cytokines have important effects on the tumor microenvironment. It has been showed that the Fibroblast Growth Factor (FGF) is closely related to epithelial-mesenchymal transformation and has important effects on osteosarcoma development and progression. In the current study, we aimed to elucidate the effect of the FGF on ADAMTS-8 expression in Saos-2 osteosarcoma model.

Material and Methods: Saos-2 cells were chosen as the osteosarcoma cell model. Cells were cultured with DMEM supplemented with 10% FCS. Cells were stimulated with 20ng/ml of FGF and incubated at different times. The effect of the FGF on ADAMTS-8 mRNA expression was determined by qRT-PCR and evaluated according to Livak method. $p \leq 0.05$ was accepted as statistically significant.

Results: FGF stimulation decreased ADAMTS-8 mRNA expression in Saos-2 cells.

Conclusion: FGF is a negative regulator of ADAMTS-8 mRNA expression in Saos-2 cells. Additional analyses should be performed to elucidate FGF-mediated transcriptional regulation of ADAMTS8 in osteosarcoma.

This study was financially supported by the Dokuz Eylul University Scientific Research Projects, Project number; THD-2023-3161.

Keywords: ADAMTS-8, FGF, Saos-2, Osteosarcoma

P147
Generation of Retinohypothalamic Tract Model Using Retina-Hypothalamus Assembloid Model for Circadian Rhythm Analysis

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Background/Aim: Circadian rhythms are essential for regulating numerous physiological processes, including sleep-wake cycles, metabolism, and immune response. The retinohypothalamic tract (RHT) plays a crucial role in transmitting light information from the retina to the suprachiasmatic nucleus (SCN) in the hypothalamus, thus synchronizing the circadian clock with environmental light-dark cycles. This study aims to develop a retina-hypothalamus assembloid model to better understand the molecular mechanisms of circadian rhythm regulation.

Materials and Methods: The generation of the RHT model was carried out in three steps: creating hypothalamus organoids, developing retinal organoids, and co-culturing these organoids from day 30 onwards to form the assembloid. The organoids were generated using human induced pluripotent stem cells (iPSCs).

Results: Hypothalamus organoids were characterized by the presence of peptidergic and hypothalamic neurons. Additionally, electrophysiological analyses performed on day 125 revealed electrical activity. Retinal organoids were characterized by their neural rosette structures, retinal progenitor cells, retinal ganglion cells, and photoreceptor progenitor cells.

Conclusion: In conclusion, the observed electrical activities, along with the characterization of retinal and hypothalamic cells, indicate that this assembloid has the potential to be used as an RHT model and for circadian rhythm research.

Keywords: Organoids, Assembloids, Retinohypothalamic Tract, Circadian Rhythm

P148
Targeting the protease BMP-1 enhances anti-tumoral effects of doxorubicin in metastatic breast carcinoma: An effect with common and distinct features

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Background/Aim: Breast carcinoma is comprised of heterogeneous groups of cells with different metastatic potential. We previously established differential features of 4T1 breast carcinoma cells metastasized to the heart (4THM), liver (4TLM), and brain (4TBM). These metastatic subsets demonstrate a cancer-stem cell phenotype inducing extensive visceral metastasis. We aimed to examine the effects of a specific bone morphogenetic protein 1 (BMP-1) inhibitor alone and in combination with doxorubicin on liver and brain metastatic murine breast cancer cells.

Materials and Methods: Using a label-free LC–MS/MS technique, we identified TGF-β1 as the top upstream regulator of the metastatic process. The free form of TGF-β1 was markedly increased in metastatic cells, potentially due to the extensive secretion of BMP-1, which releases TGF-β1 from its complex with extracellular proteins. We treated liver (4TLM) and brain (4TBM) metastatic murine breast cancer cells, as well as human breast cancer cells (MDA-MB-231), with the BMP-1 inhibitor UK383367, both alone and in combination with doxorubicin, and assessed cell proliferation and colony/spheroid formation.

Results: Treatment with UK383367 for 72 hours dose-dependently suppressed cell proliferation in both 4TBM and 4TLM cells. Lower concentrations of UK383367 were effective when treatment duration was increased fourfold. Pretreatment with UK383367 markedly enhanced the anti-proliferative effects of doxorubicin. Inhibition of BMP-1 activity decreased proliferation of MDA-MB-231 cells and enhanced the anti-proliferative effects of doxorubicin. In contrast, SB-431542, an inhibitor of TGF-β, did not enhance the anti-proliferative effects of doxorubicin. Similarly, inhibition of BMP-1 activity, but not TGF-β activity, decreased colony and spheroid formation of metastatic breast carcinoma cells.

Conclusion: Inhibition of BMP-1 activity may have therapeutic potential for the treatment of metastatic breast cancer and enhance the anti-tumoral effects of doxorubicin. These effects are not solely related to the inhibition of TGF-β1 activity and may involve other substrates of BMP-1.

This study is supported TUBITAK, Project no ; 122Z177

Keywords: breast carcinoma, BMP-1 inhibitor, doxorubicin, metastasis, TGF-¿½¿½1

P149
Evaluating Multiple Sclerosis Risk Prediction in Families Through Comprehensive Polygenic Risk Score Analysis

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Background: Multiple sclerosis (MS) is a chronic, immune-mediated, inflammatory neurodegenerative disease with a complex inheritance pattern. In our previous study, we calculated the polygenic risk scores (PRS) for six families using the 233 variants recommended by the International Multiple Sclerosis Genetics Consortium (IMSGC). While some families exhibited distinguishable PRS scores between patients and healthy relatives, others did not.

Materials and Methods: To better evaluate this, we investigated the effectiveness of PRS in predicting MS susceptibility across these families and aimed to refine current PRS models, which are predominantly based on European ancestry. We employed a comprehensive variant selection approach using Markov Chain Monte Carlo (MCMC) methods and Information Content (IC) analysis with the GINI index to re-evaluate the 233 genetic variants.

Genetic data from seven families was imputed using BEAGLE v4, with the 1000 Genomes Project serving as a reference, achieving high accuracy with an allelic R-squared cutoff of 0.90. Subsequent analyses identified variants with high predictive accuracy through a combination of Euclidean distance metrics and MCMC sampling, uncovering novel variants potentially overlooked in prior genome-wide association studies (GWAS). The methodology included a detailed imputation process, variant scoring based on effect size and zygosity, and the selection of informative subsets using MCMC Metropolis-Hasting algorithms.

Results: The results highlight eighteen unique variants, chosen from 180 shared variants that explain the phenotypes of all individuals within six MS families. Moreover, 27 variations among the 233 suggested by IMSGC explain all individual phenotypes in these families, demonstrating the importance of population-specific variant identification and the need to incorporate diverse genetic backgrounds in PRS development for improved MS risk stratification.

Conclusion: This approach re-evaluates genetic variants and identifies new ones that may enhance the predictive power of PRS models in diverse populations.

Keywords: Multiple Sclerosis, GWAS, Familial, Variant Selection, Genetic Risk Score

P150
Crosstalk Between Schwann Cells and Breast Cancer Cells: Implications for Tumor Progression and Therapeutic Strategies

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Background/Aim: Schwann cells are fundamental support cells of peripheral nervous system, known to become activated not only in nerve damage but also when encountering cancer cells. However, the impact of active Schwann cells on cancer progression remains unclear. This study aimed to investigate interactions between Schwann cells and metastatic/non-metastatic breast cancer cells.

Materials and Methods: Primary Schwann cells (naive) obtained from siatic nerves of Balb-c mice were exposed to conditioned media (CM) from metastatic (4TBM and 4TLM) and non-metastatic (67NR) breast cancer cells (activated). Subsequently, breast cancer cells were cultured in the presence of active Schwann cell-CM. Schwann cells and cancer cells were also co-cultured. The effects on proliferation were evaluated using CCK-8 assay, and cytokine responses were assessed via ELISA.

Results: Naive Schwann cell CM notably reduced proliferation of highly aggressive 4TLM cancer cells. CM from activated Schwann cells increased secretion of inflammatory cytokines such as IL-6 and MIP-2 from cancer cells. 4TLM, 4TBM, and 67NR cells secreted minimal levels of IL-6. Meanwhile, Schwann cells exhibited a basal IL-6 secretion which increased proportionally with cell seeding density. Interestingly, co-culture of 4TLM cells with Schwann cells led to a significant decrease in IL-6 secretion from Schwann cells. When control Schwann cells were cultured with 67NR cells, IL-6 levels increased significantly. Schwann cells exposed to 67NR-CM secreted more IL-6 compared to unstimulated Schwann cells.

Conclusion: Our results suggest that naive Schwann cells exhibit potential anti-tumor effects by reducing proliferation of aggressive cancer cells, while activated Schwann cells promote an inflammatory milieu conducive to cancer progression. Understanding these dynamics could inform future therapeutic strategies aimed at manipulating Schwann cell responses.

This study is supported by TUBITAK, Project no ; 122Z333

Keywords: Schwann cells, breast cancer, tumor microenvironment, MIP-2, IL-6

P151
In-vitro Effects of BMP-1 Inhibition on EMT Markers and Extacellular Proteins on Metastatic Breast Carcinoma.

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Background/ Aim: Triple negative breast cancer (TNBC) is the most malignant subtype of breast cancer in which drug resistance is common. Transforming growth factor-β (TGF-β) by inducing epithelial and mesenchymal transition (EMT) and cancer stem cell population leads to multidrug resistance. Bone morphogenetic protein-1 (BMP-1) is a metalloprotease involved in release of TGF-β. Heterogeneous group of cells form carcinomas and only a small percentage can form distant metastasis. Cells that metastasize to distant organs are treatment resistant, hence new treatments should be explored.

Materials and Methods: We previously observed that level of BMP-1 in exosomes of metastatic murine breast cancer cells is higher compared to non-metastatic cells. We here used subset of 4T1 cells that were metastasized to liver and the brain (4TLM and 4TBM respectively). These cells are highly aggressive and have both common and distinct phenotypical features as reported before. We found that inhibition of BMP-1 activity decreases proliferation of 4TLM and 4TBM cells 72 hours after treatment. We wanted to evaluate the chronic effects of BMP-1 inhibitor on metastatic potential of breast carcinoma cells.We used a BMP-1 selective inhibitor called UK383367. The 4TBM and 4TLM cells were treated with various concentrations of the inhibitor for 6-8 weeks and changes in vimentin and TWIST-1 levels were determined using western blot. Vimetin and TWIST-1 are considered as markers and mediators of EMT. Since BMP-1 is involved in extracellular matrix deposition, changes in extracellular matrix proteins were also determined.

Results: Chronic treatment with BMP-1 inhibitor markedly suppressed TWIST-1 expression in both cell lines. We,however, didn't observe any changes in secretion of extracellular proteins. Specifically, levels of trombospondin-1, collagen VA1 and laminin beta-1 wasn't changed following long-term treatment with BMP-1 inhibitor.

Conclusion: Inhibition of BMP-1 activity may revert EMT phenotype and enhance chemosensitivity of the metastatic breast carcinoma.

This study is supported by TUBITAK, Project no: 122Z177

Keywords: Bone Morphogenetic Protein 1, Metastatic Breast Cancer, EMT, extracellular matrix

P152
Phenotypic Effects of Gelatin as an Alternative to Poly-D-Lysine in Schwann Cell Culture; Possible Changes in Stimulation by Exosomes of Cancer Cells

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Introduction: Schwann cells are glial cells responsible for the coating and protection of peripheral nerve fibers. Schwann cells are protective in certain pathologies, while they may have a critical role in pathogenesis in diseases such as cancer. Primary Schwann cell culture is commonly used to explore the links between these diseases and Schwann cells.

Aim: Poly-D-lysine (PDL) is commonly used to enable Schwann cells to adhere to the surface in two-dimensional culture environments. However, gelatin is used in three-dimensional Schwann cell culture protocols. Gelatin is a more affordable alternative to PDL and is not conventionally used in two-dimensional culture models. Hence, we aimed to determine the effectiveness of gelatin in a two-dimensional culture.

Method: The sciatic nerves of mice were incubated in Schwann cell medium for 5 days, then treated with collagenase to eliminate fibroblasts. The sciatic nerves were then physically dissected and transferred to gelatin or PDL-coated dishes with Schwann medium containing Heregulin and Forskolin. As the cells progressed during culturing, medium samples were collected periodically and cytokines and chemokines such as IL-6 and MCP-1 were analyzed by the ELISA method.

Results: IL-6 and MCP-1 levels were increased in the groups stimulated with tumor mediums of 4 TLM, 4 TBM, and 67NR breast cancer cell lines in gelatin-coated dishes. Minocycline, a tetracycline antibiotic, is known to inhibit glial cell activation. Levels of IL-6 and MCP-1 secreted by Schwann cells upon stimulation with conditioned mediums of cancer cells were decreased following minocycline treatment. The results are in correlation with our previous studies in PDL-coated dishes.

Conclusion: The fact that the results we obtained on gelatin-coated dishes were in parallel with the conventional PDL-coated dishes, suggests that gelatin can also be used in two-dimensional Schwann cell culturing. Results will guide the use of gelatin as an alternative for new disease models *in vitro*.

This study is supported TUBITAK, Project no:122Z333

Keywords: cancer, cell culture, Schwann cell, gelatin, poly-d-lysine

P153
Signaling Pathways Activated by the Orphan G Protein-Coupled Receptor GPR37

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Aim: G Protein-Coupled Receptor 37 (GPR37), also called Parkin associated endothelin-like receptor, is an orphan G protein-coupled receptor associated with Parkinson's disease neuropathology. Previous studies suggested that GPR37 is activated by prosaptide TX14(A), a synthetic peptide derived from the neurotrophic sequence of prosaposin. Prosaptide TX14(A) is therefore proposed as an endogenous ligand of GPR37. However, due to numerous discrepancies associated with pairing of proposed ligands with the receptor, GPR37 remains an orphan receptor. Here, the aim of the study is to examine the signaling pathways activated by GPR37.

Materials and Methods: tGFP-tagged Human G protein coupled receptor 37 plasmid and control plasmid pCMV6, mammalian vector w/C terminal tGFP tag, were purchased from Origene (Rockville, MD). HEK293T cells were transiently transfected with GPR37 encoding plasmids using PEI MAX. Transfection efficiency was assessed via flow cytometry and fluorescence microscopy. Cell viability and cytotoxicity analyses were performed with prosaptide TX14(A) concentration varied between 0.1 µM to 1 µM. GPR37 localization with and without prosaptide treatment was observed using confocal microscopy. Western blot analysis will be performed to investigate potential changes in the PI3K-AKT, mTOR signaling pathways induced by prosaptide TX14(A).

Results: Assessment of transfection efficiency with flow cytometry and fluorescent microscopy demonstrated that HEK293T cells had a high transfection efficiency for GPR37 plasmids. While GPR37 is localized on the cell surface, it is predominantly found in the endoplasmic reticulum or Golgi apparatus; however, treatment with prosaptide induced robust endocytosis of GPR37. Further analysis will determine if prosaptide TX14(A) affects the PI3K-AKT and mTOR signaling pathways.

Conclusion: This study examines the role of prosaptide TX14(A) in GPR37 signaling to understand its role in receptor function and potential therapeutic approaches for Parkinson's disease.

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Keywords: Orphan G Protein- Coupled Receptor, GPR37, Cell Signaling, Prosaptide

P154
INVESTIGATION OF CANCER STEM CELL MECHANISM IN 3D- A549 TUMOROIDS

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Aim: The aim of the study is aimed to investigate the mechanisms of cancer stem cell development in 3D lung tumors and to discover new markers that can be used in the diagnosis and treatment of NSCLC.

Materials and Methods: A549 (lung adenocarcinoma) cells cultured in 2D; 10,000 cells were seeded in each well of the agarose-coated 96-well plate. Cells were grown in DMEM/F12 supplemented with 10% FBS and 1% antibiotics and incubated at 37 °C containing 5% CO_2 . After the tumors were grown, they were incubated at 37 °C in a hypoxic chamber containing (1 % O_2) and for 72 h. Cancer stem cell development was induced by growing the developed lung tumoroids in a hypoxic microenvironment. Whether the hypoxic condition occurred or was not analyzed by qPCR, Immunoblotting and Immunohistochemistry. Total and cell membrane proteins were then isolated from these tumor samples using the biotinylation method (A44390) and LS/MS, analysis was performed. MS analysis, raw data files were processed using Progenesis QI (v4.1; Nonlinear Dynamics) and UNIPROT Homo sapiens taxonomy was used as the search database.

Results: As a result of MS of total and membrane proteins; A total of 17 different proteins were detected, 9 and 8, respectively. Twelve of these proteins had potential prognostic significance for various cancers. Three of the proteins were prominent in the hypoxic condition.

Conclusion: As a result of the analysis, important data was obtained. It has been reported in the literature that Zinc finger CCCH domain-containing protein 13, which is more expressed especially in normoxic conditions, plays a role in the pluripotency of embryonic stem cells (ESCs). These identified proteins need to be explored through further studies.

Keywords: CANCER STEM CELL, LUNG CANCER, CELL MEMBRANE PROTEIN, PROTEOMIC

P155
Secretomes of Neuregulin-activated Schwann cells carry many common features with breast carcinoma cells.

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Background/Aim: Schwann cells are glial cells responsible for the protection and repair of axons. Primary Schwann cell culture requires specific growth factors such as neuregulin-1 and forskolin for activation and proliferation. Schwann cells activated by factors released from cancer cells resemble reactive Schwann cells and may enhance cancer growth and metastasis. Here, we examined the possible similarities and differences between cultured primary Schwann cells and metastatic and non-metastatic breast carcinoma cells using a proteomic approach.

Materials and Methods: Primary Schwann cell cultures were prepared from the sciatic nerves of adult Balb-c mice. Primary breast cancers formed by either 4TLM (liver metastatic), 4TBM (brain metastatic), and 67NR (non-metastatic) cells were cultured in vitro, and conditioned mediums (CM) were obtained in serum-free medium. Lyophilized CMs were dissolved with 8M urea in 0.1M Tris-HCl solution. Proteins were digested using the FASP method and desalted with ZipTip. Peptides were analyzed using the Orbitrap Fusion mass spectrometry instrument coupled with the Dionex Ultimate 3000 RSLCnano system. For bioinformatics analysis, the Proteome Discoverer (PD) program was used. Precursor mass tolerance was set to 20 ppm and fragment mass tolerance to 0.5 Da. The Perseus program was used for the statistical calculations of normalized abundance values.

Results: As the Venn diagram shows, of the 446 identified proteins, 363 were also found in metastatic and non-metastatic breast cancer cells. This was surprising given the differences in cell lineages. Analysis of the 363 common proteins by KEGG pathways revealed several important carcinoma-associated pathways, such as the HIF-1 signaling pathway. The 37 proteins unique to Schwann cells pointed out two reactome pathways: Regulation of Insulin-like Growth Factor transport and post-translational protein phosphorylation.

Conclusion: These preliminary findings demonstrate the need for detailed studies to explore the common and distinct features of neuregulin-activated Schwann cells and breast cancer cells.

This study is supported TUBITAK, Project no ; 122Z333

Keywords: neuregulin-1, Schwann cells, breast carcinoma, proteomics, metastasis

P156
Matrix Metalloproteinase-2 Expression Levels In Major Depressive Disorder: Can It Be A Biomarker?

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Background: Research from the literature suggests that because of their proteolytic actions on the extracellular matrix (ECM), matrix metalloproteinases (MMPs) actively contribute to major depressive disorder (MDD). By adjusting the dynamic shape of the extracellular matrix, MMP-2 plays a role in a number of physiological processes including inflammation, angiogenesis, synaptic plasticity, and tissue remodeling. It also promotes tissue homeostasis and regeneration. Increased MMP-2 expression may have a role in angiogenesis, neural plasticity, and tissue deterioration, all of which can accelerate the development of mental illnesses. Significant abnormalities are caused by neurodegenerative and inflammatory processes in people with MDD. Here, we examined the levels of MMP-2 gene expression in major depressive disorder patients against healthy controls (HC).

Materials and Method: We investigated MMP-2 expression levels in RNA samples extracted from blood samples of individuals with MDD (n=20), HC (n=19) using real-time quantitative PCR (RT-qPCR). Blood samples were obtained from the individuals with MDD patients and HC, diagnosed according to Diagnostic Statistical Manual-5 (DSM-5) criteria. Statistical analysis included Mann-Whitney U test and t-test anaylsis to examine relationships between diagnosis and MMP-2 expression levels. This work was supported by grants from the Inonu University Research Fund (#FBA-2022-2914 to CA) and the Turkish Health Institutes Presidency (TUSEB 31044 to ZK).

Results: The MMP-2 gene expression level did not significantly differ between the groups of people with major depressive disorder and healthy controls in the analyses that were conducted ($p>0.05$, $p = 0.885$).

Conclusions: According to our research, there are no appreciable variations in the MMP-2 gene's expression levels between major depressive disorder patients and healthy controls. Despite being the first research in the Turkish community, these results might only provide a rough understanding of how the MMP-2 gene functions in major depressive disorder. Changes in patient variety and the biomarkers investigated can lead to more statistically significant results and more effective outcomes overall.

Keywords: Matrix Metalloproteinase-2, Major Depressive Disorder, Extracellular matrix, Gene expression, Synaptic plasticity

P157
Role of Glioblastoma-Conditioned Media in Inducing Endothelial-Mesenchymal Transition in Endothelial Cells

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Background/Aim: Glioblastoma is an aggressive and invasive brain tumor with limited therapeutic options. The blood-brain barrier (BBB) is a significant obstacle for the development of brain-specific therapeutics, as it restricts the passage of many chemicals into the brain. Additionally, the entire profile of the endothelial cells alters in the disease conditions. Given the many unknowns about endothelial cells during glioblastoma, our objective is to understand the impact of cancer cells in the brain on endothelial cells using key EndMT biomarkers. This study investigates the endothelial-mesenchymal transition (EndMT) of human umbilical vein endothelial cells (HUVECs) exposed to conditioned medium from U87 glioblastoma cells.

Materials and Methods: HUVECs were cultured and exposed to U87 glioblastoma cell-conditioned media at various concentrations (0%, 25%, 50%, 100% dilutions with fresh media). EndMT induction was assessed
over 24, 48, and 72 hours. Immunostaining for α-Smooth Muscle Actin (SMA) and Ve-Cadherin was performed, and quantitative PCR was used to analyze the expression levels of Vimentin, Matrix Metalloproteinase-9 (MMP9), and Occludin. Fluorescence microscopy and image analysis were used to quantify marker expressions.

Results: The conditioned media caused a significant increase in mesenchymal markers, including SMA,
Vimentin, and MMP9, and a notable decrease in endothelial markers such as Occludin and Ve-Cadherin as observed by immunofluorescence staining. The tumor microenvironment's role in promoting EndMT is demonstrated by differential expression of Ve-Cadherin and SMA across various concentrations of glioblastoma-conditioned media. Our results indicate effective EndMT, characterized by a reduction in endothelial markers and an increase in mesenchymal markers.

Conclusion: This work, along with the future explorations of various secreted factors by the glioblastoma cells
on the endothelial cells, enhances our understanding of glioblastoma interactions with vasculature cells and highlights the molecular alterations involved.

Keywords: Glioblastoma, Metastasis, Endothelial Mesenchymal Transition, Biomarkers, HUVECs

P158
Single Cell Analysis of Alternative Splicing Events During Human Preimplantation Embryonic Development

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Background/Aim: During the early stages of human embryo, the chromatin is subject to an uncanny orchestration leading to fast-paced changes on gene expression patterns. Even though we now have robust knowledge on the expression levels of genes during preimplantation, how alternative splicing (AS) events lead to different transcript isoforms at this stage remains largely unexplored.

Materials and Methods: In this study, we analysed exon inclusion/exclusion events at single-cell level using a previously published dataset (GSE71318). We analysed at least three independent cells from oocyte, zygote, 2-cell, 4-cell, 8-cell, morula, early blastocyst, middle blastocyst and late blastocyst embryos as well as ICM and TE stages. We checked common alternative splicing events that only occur for each stage by employing the SCATS bioinformatics approach. Read counts for each exon were fitted onto a hierarchical model, and the differential exon inclusion levels between different cell groups were evaluated using a maximum likelihood ratio test.

Results: We demonstrated that there are stage-specific and distinctive AS events throughout human preimplantation. Highest number of stage-specific AS events was detected for the zygote stage (2506 events) followed by 2-cell (1908 events) and oocyte (1710 events) and 4-cell stages (1624 events). Genes related to histone modification, methylation and chromosome organization were consistently enriched for each phase. RNA processing genes were particularly enriched for every stage from 2-cell stage to morula. Intriguingly, genes such as AMD1 (SAM metabolism), ARMC8 (cytoskeleton organization), ATP6V1C2 (ATPase), TDP1 (DNA Repair), THOC2 (RNA transport), UBAP2L (ubiquitin-proteasome system) exhibited distinct AS events for every particular preimplantation phase.

Conclusion: Highlighting the zygotic stage as the most AS-driven phase, the comprehensive list we are providing here involves many crucial genes related to chromatin regulation and RNA processing. Understanding the functional roles of these AS events in future could provide invaluable insights that can be harnessed for developmental biology and regenerative medicine.

Keywords: Human preimplantation, embryonic development, alternative splicing, exon, bioinformatics

P159
Regulation of AKT/mTOR Pathway by RIG-I in Human Microglia under Hypoxic Conditions

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Background/Aim: Hypoxia is insufficient oxygen levels in cells or tissues due to reduced oxygen availability. The brain is particularly vulnerable to hypoxic conditions because of its high energy requirements. Therefore, hypoxic insults such as cardiac arrest, vascular catastrophe, poisoning, drowning or head trauma can result in irreversible brain injuries. Microglia are innate immune cells that reside in the central nerves system (CNS) and they become activated under hypoxia. Activated microglia release inflammatory mediators causing neuroinflammation that can eventually contribute to neuronal damage and impaired brain function or produce anti-inflammatory neuroprotective factors that help tissue repair. Retinoic-acid inducible gene I (RIG-I) is a crucial pattern recognition receptor (PRR) in cytoplasm that incites innate immune responses against viral infections. The purpose of this study was to investigate the regulatory roles of RIG-I in human microglia-like cells under hypoxia.

Materials and Methods: Human microglial cell line HMC3 was stimulated with $CoCl_2$ to induce chemical hypoxia and RIG-I was significantly downregulated by utilizing siRNA targeting RIG-I. Subsequently, co-stimulatory molecules CD80 and CD86 expressions were detected by flow cytometry while HIF-1α, a hypoxia biomarker, and downstream effectors including AKT, P-AKT, mTOR and P-mTOR protein levels were investigated by western blot.

Results: HIF-1α expression was elevated at protein level by $CoCl_2$ stimulation in microglia, validating the successfully induced chemical hypoxia. Moreover, CD80 and CD86 expressions were upregulated, indicating the microglial activation. Under chemical hypoxia, AKT and mTOR phosphorylation was significantly decreased. However, this inhibition was partially restored by the siRNA-mediated downregulation of RIG-I.

Conclusion:
Increased levels of phosphorylated AKT and mTOR through the downregulation of RIG-I, suggests a negative regulatory role of RIG-I in AKT/mTOR pathway under hypoxia in microglia.

Keywords: Microglia, Hypoxia, RIG-I

P160
Neuronal Differentiation of Mesenchymal Stem Cells in 3D Environment

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Understanding the mechanisms underlying neurodegenerative diseases is critical for the development of effective therapeutic approaches. Despite advancements in the field, current disease and tissue modeling systems remain insufficient in replicating *in vivo* conditions accurately. The sensitivity and limited lifespan of neurons, coupled with the absence of a complex brain environment, pose significant challenges in brain modeling. To address these limitations, the combination of stem cell technology and three-dimensional (3D) bioprinting presents a promising approach for mimicking physiological brain conditions. In this study, bone marrow-derived mesenchymal stem cells (bm-MSCs) were bioprinted using a gelatin-based hydrogel matrix. These bioprinted tissues were cultured for seven days in a specific medium containing several inducers for neuronal differentiation. The constructs were subsequently analyzed for cell viability and neuronal morphology. Furthermore, the effect of glial cells on neuronal differentiation were investigated through the co-culture of primary astrocytes and bm-MSCs, as well as by varying the ratios of astrocyte-conditioned medium to neuronal differentiation medium. Both two-dimensional (2D) well plate and 3D bioprinting experiments were conducted and analyzed for morphology and cellular viability, respectively. The results demonstrated that MSCs exhibited high cellular viability within the 3D bioprinted constructs and showed a propensity to differentiate into neurons. Additionally, the 1:1 mixture of astrocyte-conditioned medium and neuronal differentiation medium yielded cellular viability comparable to control groups. These findings offer a valuable approach for neuronal differentiation and hold promise for further studies in physiologically relevant modeling brain diseases using stem cell and 3D bioprinting technologies.

Keywords: stem cells, neuronal differentiation, 3D Bioprinting

P161
The Effect of Histone Chaperone YL-1 Knockdown in Epithelial to Mesenchymal Transition

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Background/aim: The epithelial-to-mesenchymal transition (EMT) is a developmental process where epithelial cells lose their polarity and cell-cell adhesion, resulting in unpolarized and motile properties. A true EMT is marked by the downregulation of E-cadherin and the expression of N-cadherin. Histone deposition complexes control the placement of histone variants at specific chromatin regions, with histone chaperones playing a crucial role. YL-1, a particular histone of deposition-and-exchange chaperone for H2A.Z, plays a key role in various nuclear processes and transcription, including gene activation and silencing. However, there is limited research on how this regulation is achieved, particularly regarding the role of chaperones. Our main objective is to analyze the epigenetic regulation of EMT by the histone variant H2A.Z's chaperone YL-1.

Materials and methods: MCF10A cells were transfected with siRNA targeting YL-1 or a control siRNA using Lipofectamine RNAi Max. Total RNA was isolated from both cells, and EMT-related markers were analyzed using qPCR, and immunostaining. Total RNA was used for RNA sequencing, and expression profiles were analyzed bioinformatically, including gene ontology enrichment. Cell cycle analysis was performed on the same samples.

Results: Our results revealed that the depletion of YL-1 resulted in an EMT-like phenotype, characterized by the downregulation of epithelial markers (such as CDH1) and the upregulation of mesenchymal markers (such as FN1 and CDH2). Differentially expressed genes obtained from RNA sequencing data revealed the enrichment of gene sets indicating EMT. Specifically, wound healing, extracellular matrix organization, epidermal cell differentiation, skin development, and keratinization have all been implicated in the development of EMT.

Conclusion: Our results show that the downregulation of histone variant chaperone YL-1 leads to a significant EMT-like phenotype that is accompanied by the downregulation of CDH1 gene expression. This gives promising insight for further research.

This study is supported by The Scientific and Technological Research Council of Turkey (TUBITAK) with grant numbers 118C354 and 122Z215.

Keywords: EMT, YL-1, MCF10A, RNA-seq

P162
Interplay of Elf3 and DNA Methylation Enzymes in Mesenchymal Epithelial Transition

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Background/Aim: The mesenchymal-epithelial transition (MET) is a biological process that plays an important role in various essential physiological and pathological events. Recent findings suggest that MET is not simply the reverse of Epithelial-mesenchymal transition (EMT). The protein Elf3 plays a crucial role in regulating MET; its absence during MET results in an altered MET observed by the aberrant localization of E-cadherin. DNA methylation, a significant epigenetic modification facilitated by DNA methylation enzymes, is essential in defining cellular identity. Close examination of the expression pattern of DNA methylation enzymes during EMT and MET, and analysis of their promoters suggested a regulatory role of Elf3. The objective of this study was to explore the potential interplay between DNA methylation enzymes and Elf3, which is crucial during MET using an integrated approach, encompassing gene modulation, DNA-protein interaction studies, and bioinformatics.

Materials and Methods: We studied the interplay of Elf3 and DNA methylation enzymes in MET using siRNA Elf3 in NMuMG cells. EMT-related mRNA gene expression profiles were determined by qPCR. Using RNA-Seq technology, the transcriptional effect of Elf3 absence in MET were shown. Promoter constructs were prepared for reporter assay and promoter enrichments of DNA methylation genes were tested with ChIP assay.

Results: The depletion of Elf3 during MET results in a downregulation of Dnmt1, Dnmt3b and Uhrf1. Furthermore, analysis of the promoter sequence of all three genes revealed evolutionary conserved Elf3 binding sites. Elf3 was able to induce the promoter of Dnmt1, Dnmt3b and Uhrf1 in luciferase reporter assays and was enriched on the promoters of DNA methylation enzymes during MET.

Conclusion: Findings revealed an interplay between Elf3 and DNA methylation enzymes, expanding on its known activities in MET regulation and unveiling new ones.

This study is supported by TUBITAK with grant number 120N556 and 122Z886.

Keywords: MET, DNA methylation, Elf3

P163
The Relationship between Chemically Induced Oxidative Stress in Organelles and, UPR in Flaveria Species with Different Carboxylation Pathways

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Background/Aim: *Flaveria* genus is often used in C_4 studies, as it has species with varying levels of C_4 evolution. However, there is no comparative study of this genus in terms of endoplasmic reticulum (ER) stress. ER stress is very important in oxidative stress because it results from the accumulation of reactive oxygen species in organelles. ER stress was created by treatment chemicals that trigger organelle-specific oxidative stress to *Flaveria robusta* (C_3), *Flaveria anomala* (C_3-C_4) and *Flaveria bidentis* (C_4) species with different carboxylation pathways. The results added a further level of complexity that we hope will reveal differences in the UPR.

Materials and Methods: Methyl viologen (MV), rotenone (ROT), and 3-Amino-1,2,4-triazole (3-AT), which respectively cause oxidative stress in chloroplasts, mitochondria, and peroxisomes, were applied to leaf discs. The degree of oxidative damage was measured by CAT and POX assays. Differences in the expression of UPR-related genes between species were detected by qRT-PCR.

Results: CAT activity was decreased in all three species with MV and 3-AT treatment. The POX activity of C_4 decreased with MV. Under ROT treatment, CAT activity increased highest in C_3-C_4. According to the expression of UPR genes respectively of resistance to ER stress is C_3, C_3-C_4 and C_4.

Conclusion: Our results support the hypothesis that C_2 photosynthesis is a unique type of photosynthesis, rather than an intermediate form between C_3 and C_4 types. Induction of oxidative stress in different organelles led to different responses. This shows that the location of oxidative stress is important. Finally, the C_4 species our study was overall the most sensitive type, supporting the idea that C_4 species aren't necessarily more tolerant than C_3 species, but rather they are a unique evolution to adapt to photorespiration.

Keywords: Flaveria, Photorespiration, Endoplasmic Reticulum ER Stress

P164
Generation of Connexin26 Knock-Out HaCaT Keratinocyte Cells Using CRISPR/Cas9

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Background / Aim: The epidermis, the outermost layer of the skin, is an avascular tissue mainly composed of keratinocytes. As the skin lacks blood vessels, communication between keratinocytes is facilitated by gap junctions (GJs) to maintain epidermal homeostasis. Six connexins form connexons, or hemichannels. Docking of hemichannels between neighboring cells assembles GJs, enabling small molecule transport. Within the epidermis layers, connexins show distinct expression patterns. Cells in the spinous layer primarily express Connexin26 (Cx26) encoded by the *GJB2* gene. Mutations in the *GJB2* are linked to various skin diseases including autosomal dominant Keratitis-Ichthyosis-Deafness (KID) syndrome. KID syndrome causing Cx26-D50Y and G45E mutations leads to hyperactive hemichannels and uncontrolled molecule trafficking disrupting homeostasis. Moreover, D50Y and G45E mutations show mild symptoms and lethality in patients, respectively and the underlying mechanisms of these phenotypes with varying severity are unclear. Examining the effect of each mutation requires model systems that lack endogenous Cx26 expression and accurately reflect physiological processes in the epidermis. Therefore, employing human keratinocyte cells is important as they provide more accurate representation of epidermal physiology. The presence of endogenous Cx26 expression in keratinocytes can complicate the investigation of aspects of exogenously expressed mutant Cx26 proteins. Thus, the aim of the study is to knock out the Cx26 gene in HaCat cells to create a model system. **Materials and Methods:** The CRISPR/Cas9 technique is used to edit HaCaT cells. Insertion of selected gRNAs into Lenti-CRISPR-V2 vector will be verified with PCR and sequencing. Then, viruses produced using gNRA vector will be used to infect the cells. Expression analysis using q-RT-PCR and Western blot will confirm the editing. **Results**Selected gRNAs were annealed and cloned into Lenti-CRISPR-V2 vector. Insertion of gRNAs was validated with PCR and sequencing. **Conclusion**: Employing the Cx26 knock-out HaCat cells to examine the phenotypic effects of the mutations will advance our knowledge about the mechanism of action.

Keywords: connexin, epidermis, mutations, KID syndrome, CRISPRCas9

P165
Evaluation of the Role of Serum HN1L as a Biomarker in the Early Diagnosis of Breast Cancer

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Background/Aim: Hematologically and Neurologically Expressed 1-Like (HN1L) protein is known to be characterized by metastasis and cancer stem cell differentiation. When the few studies in the literature on the role of HN1L in plasma and tissue cancer samples are evaluated as a whole, it is predicted that it may be a potential biomarker candidate in the early diagnosis of cancer. For this purpose, serum HN1L levels in the blood of healthy individuals and newly diagnosed breast cancer patients were investigated for the first time.

Materials and Methods: Serum HN1L levels of healthy individuals (n=10) and patient individuals (n=24) were measured using the Enzyme-Linked Immunosorbent Assay (ELISA) method following the manufacturer's HN1L kit protocol (Abbexa, Cambridge, United Kingdom). Statistical significance was evaluated at p-value <0.05.

Results: According to the findings of the study, the mean serum HN1L levels of healthy individuals (0.25 ng/ml) were higher than the mean of patient individuals (0.02 ng/ml). Statistically, no significant difference was observed between the mean values of both groups (p=0.07).

Conclusion: When evaluated with the clinical data of the patients, low serum HN1L level may be a potential biomarker candidate for the early diagnosis of breast cancer. However, it is thought that the obtained data should be confirmed in a larger population.

The study was supported by Bursa Uludag University Scientific Research Projects Coordination Unit (BAP) (Project number: TGA-2022-738)

Keywords: Breast Cancer, HN1L, Biomarker, Early Diagnosis, Cancer Stem Cell

P166
Analysis of Inflammasome-Related Innate Immunity Gene Variants in Ancient Human Genomes

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Background/Aim: Polymorphisms are one of the main factors determining the immune response of the host to a pathogen. Sequences inherited from Neanderthals can have functional effects such as conferring resistance to AIDS or susceptibility to COVID-19. Inflammasomes are cytosolic protein complexes of the innate immune system specialized in the recognition of pathogens and their clearance. To date, there is no such studies on the inflammasome-related genes. The aim of this project is to identify structural and functional differences of inflammasome-related innate immunity genes between modern and archaic humans.

Materials and Methods: Publicly available Neanderthal and Denisovan genome databases were screened to identify variations in inflammasome-related innate immunity genes. Conservation analyses among various species and different modern human populations were carried out. To assess the impact of the variation on the stability of the protein and its function, we transfected the "modern human" and "archaic" sequences of a selected gene in HEK293FT cells and evaluated their expression and innate immune response to pathogens through Western blotting and lactate dehydrogenase assay.

Results: We analyzed twenty-seven inflammasome-forming and inflammasome-related genes and identified eighteen non-synonymous variations in ten different genes. Most of the variants were conserved among other species suggesting an important functional role for these residues. For functional analysis, we first selected a gene presenting a Denisovan variation in proximity of a cleavage site leading to the activation of the coded protein. Preliminary wet laboratory results suggest that the archaic variant might influence the function of this protein.

Conclusion: Overall, these results demonstrate that inflammasome-related genes underwent non-synonymous variations during evolution. Whether these variations shaped the response to pathogens must be determined. Analyzing the genome of our closest evolutionary relatives will give insight into new functions of innate immune genes.

Keywords: Archaic Human, Innate Immunity, Inflammasomes, Pyroptosis, Evolution

P167
Surface Plasmon Resonance as a Powerful Tool for Identifying Molecular Interactions of Variable Heavy-chain Domain of Heavy-chain-only Antibodies

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Background/Aim: The identification and characterization of protein-protein interactions are fundamental to understanding biological processes and developing therapeutic agents. Surface Plasmon Resonance (SPR) is a powerful technique for real-time, label-free detection of molecular interactions. This study leverages SPR to investigate the binding properties of several distinct Variable Heavy-chain domains of Heavy-chain-only Antibodies (VHHs), each targeting different types of proteins such as membrane receptors and transcription factors derived from different hosts. We first aimed to identify the on- and off-rates of the VHHs. Subsequent epitope binning and competition assays provided insights into their functionality.

Materials and Methods: We employed SPR to determine the dissociation constant (KD) values for these VHHs, providing insight into their binding affinities. Additionally, epitope binning assays were conducted to elucidate the binding epitopes and competition assays to understand the competitive interactions among the VHHs. SPR experiments were performed on a Biacore T200. All VHH sequences were obtained from either an immunized llama or a synthetic yeast display library. VHH encoding cDNAs were expressed in BL21 derived bacterial strains.

Results: Using multi-cycle kinetics, we obtained a broad range of affinities (picomolar to micromolar). Epitope binning assays further classified the binding sites, demonstrating distinct epitope regions targeted by each VHH. Competition assays provided deeper insights into the potential overlapping and non-overlapping binding regions. Our results demonstrate that SPR is an effective tool for precise measurements of VHH interactions.

Conclusions: In conclusion, SPR results have provided valuable insights into on- and off-rates of the VHHs indicating their occupancy time of their targets using multi-cycle kinetics. These analyses have highlighted the important correlation between the off-rates and KD values. Additionally, epitope binning and competition assays have further classified the nanobodies, revealing distinct epitopes targeted by each VHH. Together, these findings reinforce the utility of SPR in nanobody characterization and development of the nanobodies as therapeutics.

Keywords: surface plasmon resonance, epitope binning, binding affinity, kinetic analysis

P168
Regulation of the Innate Immune Response by a Specific SARS-CoV-2 Protein

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Background/Aim: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a coronavirus causing Covid19 that alters the cellular machinery to replicate its own genetic material within the host cell's cytoplasm. As the first line of defense against infection, the innate immune response restricts almost every stage of the virus life cycle and upregulates interferon-stimulated genes (ISG) expression to help eliminate viruses. SARS-CoV-2 has many counterstrategies that boost replication and transmission, while trying to suppress interferon response. Our project aims to determine whether the innate immune response triggered by SARS-CoV-2 is mediated through inflammasome complexes and to elucidate the molecular mechanism of this interaction.

Materials and Methods: SARS-CoV-2 proteins that activate inflammasomes were identified by transfecting them individually in HEK293FT cells, ectopically expressing inflammasome components and by monitoring hallmarks of inflammasome activation. The identified protein was transduced in macrophages and endogenous inflammasome activation was monitored. To understand the molecular mechanism, subcellular fractionation and qPCR analysis were performed in viral protein transfected cells.

Results: Our experiments demonstrated that when overexpressed in HEK293FT cells reconstructed with different inflammasome components, a new SARS-CoV-2 protein could activate inflammatory response characterized by IL-1beta secretion, Caspase-1 cleavage and lactate dehydrogenase release. Moreover, we observed an increase in ASC speck formation when this protein was overexpressed in HEK293FT ASC-GFP stable lines. Mechanistically, this protein was found to regulate the expression of some inflammasome receptors and triggered a subcellular redistribution of certain inflammasome proteins.

Conclusion: Overall, these results identified an unpublished SARS-CoV-2 protein that modulates the host innate immune response through inflammasome regulation. Further analysis will be conducted to fully understand the implications of this interaction on SARS-CoV-2 pathogenesis and evaluate the impact of changes occurring in this protein on inflammasome activation.

Keywords: Innate Immunity, Inflammasomes, Pyroptosis, SARS-CoV-2, Interferon

P169
Role of Aryl Hydrocarbon Receptor Signaling and Inflammation in Leukemia Regulation via Bone Marrow Adipose Tissue Mesenchymal Stem Cells

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Background/Aim: The bone marrow (BM) is crucial for regulating hematopoietic stem cells (HSCs), primarily through various cellular components within the BM niche, including mesenchymal stem cells (MSCs). MSCs also originate from adipose tissue in the BM and are referred to as BM adipose tissue (BMAT) MSCs. Disruption of HSCs regulation leads to BM pathologies, notably leukemia. Although the pathogenesis of leukemia is not fully understood, recent studies suggest that BMAT contributes to its development via xenobiotics influencing leukemia through Aryl hydrocarbon receptor (AhR) signaling. The mechanisms remain unclear, and the role of this pathway in leukemia progression is unknown. In this context, we aimed to investigate the effects of stimulated AhR signaling in BMAT-MSCs.

Materials and Methods: Hematological pathologies such as Acute Myeloid Leukemia (AML) and Fanconi Anemia (FA) are related to abnormalities in the BM niche and thus MSCs. We investigated the effects of AhR signaling using BMAT-MSCs from HD (n=3), FA (n=3), and AML (n=3) patients were treated with 100 nM FICZ (6-Formylindolo[3,2-b] carbazole), 1.5 µM SR1 (StemRegenin1), and 100 ng/mL TNFα (Tumor Necrosis Factor-alpha) for 18 hours. HL60 cells (human promyelocytic leukemia cell line) were incubated for 24-hours with conditioned media obtained from BMAT-MSCs after this 18-hour treatment. The impact of the resulting secretory changes on HL60 cells was then analyzed using flow cytometry, examining proliferation, apoptosis, and changes in surface markers CD274, CD133, CD36, and CD11b.

Results: Our results demonstrated that AhR stimulation did not significantly impact HL60 cell proliferation and apoptosis. However, in the TNFα group, inflammation led to a notable increase in surface markers CD36 and CD11b populations.

Conclusion: Inflammatory conditions induced by TNFα can enhance CD36 and CD11b expression, suggesting a role in leukemia progression. Further research is needed to elucidate how BMAT-MSCs and inflammation contribute to leukemia pathogenesis.

This work was supported by the TUBITAK (Grant no: 220S759).

Keywords: Bone Marrow Adipose Tissue, Mesenchymal Stem Cells, Leukemia, Aryl Hydrocarbon Receptor, Inflammation

P170
Identification of Human Innate Immune Gene Homologs in Bacteria

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Background/Aim: Infectious agents exist since ancient times, and the survival of the host organism is ensured by an efficient immune system that recognizes and eliminates pathogens. Despite extensive knowledge on the immune system of mammals, bacterial immune system is less understood. In fact, bacteria can also be infected by viruses and the exact mechanisms that bacteria employ to eliminate phages remain unclear. In recent years, cGAS- and viperins-like bacterial proteins that recognize viral DNA were found in a limited number of studies, suggesting that bacteria may also have other uncharacterized receptors and sensors for pathogens. The aim of this study is to identify human innate immune gene homologs in bacteria.

Materials and Methods: Bacterial homologs were identified through a blast analysis of human sequences against bacterial genomes. Conservation of the identified homolog proteins among other species was determined. Specific primers were designed, and polymerase chain reaction was performed to verify the expression of one of the hypothetical homolog gene in the corresponding bacterial strain.

Results: Forty proteins involved in human innate immunity were analyzed. Bacterial homologs for fifteen proteins were identified. Homolog proteins were found in different bacterial species including pathology-causing *Salmonella enterica* or more archaic-type bacteria. Some of the homologous proteins were hypothetical with incomplete sequences. One of the hypothetical homolog gene was selected and its expression was confirmed in *Salmonella* Typhimurium strain by PCR. Interestingly, this gene was found to have a higher expression at the exponential phase of bacterial growth.

Conclusion: These results confirmed that proteins of the human innate immune system are preserved in a wide variety of living species including bacteria. Characterization of the full sequence of different homologs and confirmation of their function is in progress. Elucidating bacterial innate immunity will give valuable insights into the evolution of the innate immune system.

Keywords: Innate Immunity, Inflammasomes, Pyroptosis, Evolution, Bacteria

P171
Role of long-noncoding RNA NRAV on interferon response in melanoma.*

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Background/ Aim: The role of long noncoding RNAs (lncRNAs) expressed by tumor cells in controlling antitumor immune responses remains unclear. This project focuses on melanoma, a highly aggressive skin cancer, to investigate the roles of a recently identified and understudied lncRNA called NRAV (Negative Regulator of Antiviral Response) in regulating the interferon response in cancer cells. NRAV has been shown to epigenetically inhibit interferon-stimulated genes (ISG). Our-own strong unpublished preliminary data indicating that NRAV expression is significantly higher in human melanoma cells compared to various immune cells. High NRAV expression negatively impacts survival, particularly in tumors rich in immune cells. Cancers with high NRAV expression largely lack the IFNγ (Interferon gamma) response gene signature. In line with these data, our project's focus is on investigating the unknown lncRNA called NRAV in melanoma tumor immunity.

Materials & Methods:In the current phase of the project, A375 melanoma cell lines with silenced and over-expressed NRAV gene have been created using shRNA and NRAV contaning vectors via bacterial transformation and retroviral transduction. They were treated with IFNγ and IFNa to investigate the effects of NRAV manipulation on IFN response. ISG transcript levels in response to IFN were measured using qRT-PCR.

Findings: In the time-dependent IFNγ stimulation experiment, NRAV silencing under IFNγ stimulation led to a significant increase in ISG transcript levels espacially at 4th hour after treatment. Remarkably, some ISG transcript levels significantly decreased under IFNγ treatment in time-dependent manner. In concurrent experiments with IFNα, ISGs transcript levels either did not change or showed non-significant variations.

Conclusion:All these findings suggest that NRAV might play a more inhibitory role in IFNγ response compared to IFNa in melanoma. NRAV's role in melanoma and tumor immunity will be clarified through experiments focused on proliferation, localization, and identifying NRAV protein partners.

This research project is supported by TUBITAK-1001.

Keywords: NRAV, lncRNA, Melanoma, IFN Response, Immunotherapy

P172
Investigation Of The Microenvironment In Lung Cell Line

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The environmental conditions in which the cell is located affect all cell functions and intracellular and extracellular pH levels are one of these factors. These pH changes affect cell metabolism processes and some membrane proteins' functions. Changes in pH can also affect structures involved in intercellular communication. Exosomes, which carry many bioactive molecules are also known as extracellular organelles that enable information exchange between cells. pH changes affect exosome secretion. The aim of this study was to compare miRNA expression in normal and pH-exposed cells. For this purpose, WI38 cells were incubated at acidic and alkaline pH values for 24 hours, and exosomes secreted by WI38 cells were isolated. Isolation was performed using the ExoQuick-TC kit following the kit protocol. SEM, Zeta sizer were used for exosome characterization, and Exo-Check Exosome Antibody Array kit was used according to the manufacturer's instructions for surface markers. miRCURY Exosome Kit was used for miRNA isolation according to the manufacturer's instructions. Afterwards, cDNA was obtained using the miRCURY LINA RT kit and the obtained cDNA was compared using the miRCURYLNA SYBR Green PCR kit against the expressions of miR146b-5p, miR5189-3p, miR21-5p, miR21, miR145 miRNAs according to the CT ($2^{-\Delta\Delta CT}$) method. Both SEM and Zetasizer data revealed that exosome isolation from three groups of cells was successful, and the average sizes of exosomes were between 75 nm and 102.5 nm, respectively. Exosomes in each group were positive for CD63 and CD81. miRNA expressions were selectively variable according to miRNA type in cells incubated in alkaline and acidic environments compared to those incubated in normal conditions. In light of these data, it can be concluded that miRNA expression is greatly affected by the pH values of the microenvironment in which the cell is located, and this situation also affects the behavior of cells around diseased tissues.

Keywords: alkaline, acidic, exosomes, microenvironment, WI38

P173
Functional Analysis of IPP, A Gene Implicated in Intellectual Disability

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Background: Intellectual disability (ID) affects 2% of the global human population. The intracisternal A particle-promoted polypeptide (IPP) gene, a member of the Kelch-like protein family (KLHL), has been associated with ID via whole exome sequencing in two Iranian families. KLHL proteins are known to play an adapter role in the ubiquitination complex between Cullin3 and substrate proteins and participate in F-actin organization, stabilization, crosslinking, and cytoskeleton modulation. In this study, we aim to elucidate the function of IPP in neurodevelopment using *Drosophila melanogaster* as a model for *in vivo* experiments and HeLa cells for *in vitro* studies.

Materials and Methods: The plasmid carrying Gal4 downstream of the 5' UTR region of *CG9426*, the putative IPP ortholog in *Drosophila*, and the plasmids carrying HA-tagged wild-type (WT) and mutant IPP downstream of UAS were cloned to generate the CG9426-GAL4, UAS-IPP^{WT} and UAS-IPP^{G397R} lines. The expression pattern of *CG9426* was investigated using the Gal4-UAS system and immunohistochemistry. The morphology of the mushroom body was assessed in *CG9426* knockout mutant.

Results: Knocking out *CG9426* affects viability and leads to mushroom body abnormalities in *Drosophila*. These phenotypes are rescued by the introduction of the wild-type CG9426 allele and expression of human IPP. *CG9426* is expressed in a subset of glial cells in the brain of 3rd instar larvae and in the adult fly brain.

Conclusion and Future Directions: *CG9426* is the ortholog of *IPP* in *Drosophila*. Its expression in a subgroup of glial cells is essential for fly development. Future experiments will study the subcellular localization of WT and mutant IPP proteins and identify their interaction partners in Hela cells.

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Keywords: IPP, Intellectual Disability, Glia, Drosophila melanogaster

P174
Data Mining for Retrieving All Known Ciliary Genes

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Background/Aim: Cilia are essential organelles that play a role in many cellular processes, including development, and are linked to a number of human disorders. Compiling a complete list of all known ciliary genes is essential for diagnosing ciliary diseases and comprehending the molecular mechanisms behind them. The goal of this work is to create a computer pipeline for automating the process of identifying and compiling the whole list of ciliary genes.

Materials and Methods: Using terms like “cilia,” “cilium,” “transition zone,” “basal body,” and “ciliogenesis,” we created a unique search technique for each of the 19,505 human genes in order to find every relevant entries for each gene. Filtering the search results allowed for the extraction of unique PubMed IDs (PMIDs). Using “cilia localization” as a basis, we have created an updated version of the SYSCILIA gold standard (SCGSv3) of known ciliary genes.

Results: The computational pipeline that was established was able to effortlessly obtain PMIDs and particular data for every gene associated with cilia. Comprehensive gene-PMID pairings are included in the resulting data frame. This approach greatly minimizes the human effort required for literature mining while guaranteeing comprehensive coverage of the relevant information.

Conclusion: Our computational pipeline effectively searches PubMed for known ciliary genes, thus generating a complete list of known ciliary genes. Furthermore, this pipeline may be modified for different biological gene identification.

Keywords: Cilia, Data Mining, Computational pipeline

P175
Investigating the Regulatory Roles of Protein Palmitoylation During Cell Division

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Background: During cell division, cells undergo dramatic reorganizations that are mainly driven by post-translational modifications (PTMs), most prominently protein phosphorylation. However, there has been growing evidence on the roles of other PTMs and their crosstalks during cell division (Eifler et.al., 2018).
Palmitoylation is a fatty acid modification that targets the cysteine residues of proteins (Resh, 2006). As a reversible modification, palmitoylation has been shown to be important for several molecular events, including but not restricted to protein-membrane interactions, and protein-protein interactions (Linder and Deschenes, 2007).

Recently, we have discovered a cell division regulatory role for the protein palmitoylation. We showed that palmitoylation is required for the cell cycle-specific localization of Protocadherin 7 (PCDH7). Inhibiting the responsible Palmitoyl Acyltransferase (PAT) enzyme for this modification disturbs cell division and causes multinucleation (Özkan et.al. 2023). Based on these results, we proposed that palmitoylation plays a crucial role in cell division, and PCDH7 is one of the many proteins whose regulation during cell division depends on protein palmitoylation.

Material and Methods: We combined click-chemistry-based palmitoylation assay with SILAC-based quantitative proteomics to uncover the cell cycle-specific palmitoylation events. We metabolically labeled SILAC cells with a clickable analog of palmitic acid (17-ODYA). We then employed click-chemistry to incorporate the biotin into 17-ODYA-containing proteins. Palmitoylated proteins were then enriched using streptavidin, on-bead digested and analyzed in LC-MS/MS. SILAC labels were used to quantify interphase and mitosis-specific palmitoylation events.

Results: We identified numerous novel palmitoylation events specific to mitosis and interphase.

Conclusion: Our data suggest that multiple proteins are palmitoylated in a cell cycle-dependent manner and palmitoylation might be a regulatory mechanism for cell division. Further validations will be performed to understand the role of protein palmitoylation in cell division.

Keywords: PTM, Palmitoylation, Cell Division, Proteomics, LC-MSMS

P176
Evaluation of protein expression during the differentiation of monocytes to dendritic cells derived from human PBMCs

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Background/Aim: This study aims to elucidate the differentiation of monocytes, isolated from human peripheral blood mononuclear cells (PBMCs), into dendritic cells using GM-CSF and IL-4. It also seeks to assess the expression levels of the transcription factors ZEB2, ROR1, STAT6, ETS1, and the cell surface proteins Plexin C1, SOX2, and TWIST. Dendritic cells are pivotal in the immune system, performing antigen processing and presentation to initiate and regulate immune responses. Dysregulation in the differentiation pathway from monocytes to dendritic cells can compromise immune functionality and has been linked to various pathological conditions, including cancer. ZEB2 is implicated in epithelial-mesenchymal transition (EMT) and cellular differentiation; ROR1 is associated with cell proliferation and migration; STAT6 is crucial for Th2 cell differentiation and allergic reactions; ETS1 is essential for T cell maturation; Plexin C1 is involved in cellular movement and developmental processes; SOX2 is key for maintaining stem cell pluripotency; and TWIST plays a role in EMT and tumor progression.

Materials and Methods: Monocytes were differentiated into dendritic cells through treatment with GM-CSF and IL-4. The efficiency of differentiation was evaluated using flow cytometry with CD14 and CD83 markers. Subsequently, cell lysates from both monocytes and dendritic cells were prepared, and Western blot analysis was employed to quantify the expression of investigated proteins.

Results: Flow cytometry confirmed effective differentiation of monocytes into dendritic cells, characterized by appropriate expression of CD14 and CD83 markers. Western blot analysis demonstrated distinct expression profiles of the examined proteins between monocytes and dendritic cells.

Conclusion: This study provides significant insights into the roles of specific transcription factors and surface proteins during the monocyte-to-dendritic cell differentiation process. Understanding these mechanisms is crucial, as anomalies in dendritic cell differentiation can alter immune responses and contribute to disease progression, including cancer. These findings enhance our comprehension of immune regulation and cellular differentiation.

Keywords: monocyte, dendritic cell, differentiation, immune regulation, EMT

P177
Transcriptome Analysis Reveals Neuroendocrine-Like Signatures in Muscle-Invasive Bladder Cancer Expressing Nucleocytoplasmic β-Catenin

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Background/Aim: Bladder cancers exhibit significant heterogeneity, with varying molecular profiles and histopathological features, complicating their diagnosis and treatment. The molecular mechanisms underlying this heterogeneity remain poorly understood. This study aimed to elucidate the molecular landscape of neuroendocrine-like bladder tumors, particularly focusing on the role of β-catenin localization.

Materials and Methods: The study evaluated the subcellular localization of β-catenin and the expression of neuroendocrine markers, including Synaptophysin, CD56, and Chromogranin A, in muscle-invasive bladder cancer (MIBC) and small cell carcinoma of the bladder (SCCB) patients using immunohistochemical staining. Transcriptome data from 7 β-catenin negative MIBC, 22 β-catenin positive MIBC, and 3 SCCB patients were generated and analyzed. The analysis included Gene Set Enrichment Analysis (GSEA), Weighted Gene Co-Expression Network Analysis (WGCNA), Gene Set Analysis (GSA), and transcription factor binding motif analysis. The molecular classification tool was also utilized to perform a comprehensive analysis of the molecular characteristics associated with neuroendocrine-like differentiation in muscle-invasive bladder cancers.

Results: Our study revealed a significant increase in the nucleocytoplasmic localization of β-catenin in muscle-invasive bladder cancer (MIBC) patients, which was strongly associated with neuroendocrine differentiation. Immunohistochemical analysis showed that β-catenin-positive tumors had elevated levels of neuroendocrine markers, such as Synaptophysin, CD56, and Chromogranin A. We identified an enrichment of transcription factors, including YY1 and members of the E2F family, as well as components of the Polycomb repressive complex. These findings suggest that these factors are crucial in driving the neuroendocrine phenotype.

Conclusion: Our findings collectively provide novel insights into the molecular intricacies of neuroendocrine-like bladder tumors, highlighting the significant role of β-catenin localization in neuroendocrine differentiation and uncovering the contributions of key transcription factors and chromatin remodeling complexes These findings enhance our understanding of the molecular mechanisms underlying these tumors and point to new avenues for targeted therapies.

Keywords: Bladder cancer, neuroendocrine differentiation, molecular subtype, transcriptomics, Polycomb repressive complex

P178
Characterization Of MicroRNA-142 Networks Controlling Tumor Immunity in Melanoma

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Background/Aim: Melanoma is one of the most aggressive forms of skin cancer. MicroRNAs (miRNAs) are pivotal in immune responses within the tumor microenvironment by regulating target gene expression in cancer. Among these, miRNA-142 has emerged as a critical regulator of immune cell function. However, miRNA-142 has not been studied in cytotoxic T cells (CTLs) and melanoma cells for their potential roles in regulating antitumor immunity.
This study aims to investigate the role of miRNA-142 in T cells and tumor cells during antitumor immunity.

Materials and Methods: Comprehensive bioinformatic analyses were conducted using skin cutaneous melanoma (SKCM) data from the Cancer Genome Atlas (TCGA). Kaplan-Meier survival analysis was performed using TCGEx to assess the correlation between miRNA-142 expression levels and patient survival outcomes. Gene Set Enrichment Analysis (GSEA) was utilized to identify pathways enriched with miRNA-142 target genes. Correlation analysis was used to determine genes correlated with miRNA-142 in melanoma patients. Principal Component Analysis (PCA) was used to visualize the variance in gene expression profiles from different gene sets between high and low miRNA-142 expression levels, highlighting the impact of miRNA-142.

Results: Survival analysis shows melanoma patients with high miRNA-142 expression had a higher survival rate. Additionally, genes positively correlated with miRNA-142 were found to play an important role in T cell development and signal transduction. Supporting this, the expression of certain cytokines (IFNG, PRF1) involved in the immune response in T cells also positively correlated with miRNA-142. This suggests that miRNA-142 plays a role in tumor immunity in melanoma through CTL-mediated immune response. GSEA and PCA results indicate that high miR-142 expression is associated with increased activity in pathways related to immune response, inflammation, and cell signaling in melanoma.

Conclusion: Bioinformatics analyses have provided important preliminary information on how miR-142 mediates tumor immunity in melanoma. Following these findings, further experimental investigations will be conducted.

Keywords: Tumor microenvironment, microRNA-142, melanoma, cytotoxic T lymphocytes, bioinformatics

P179
Comparison of Different Chromatography Columns in Monoclonal Antibody Purification

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Monoclonal antibodies (mAbs) are crucial in diagnostic and therapeutic applications. These antibodies are produced using methods such as hybridoma technology, recombinant DNA technology, phage display, and single B cell technology. It is essential to purify these monoclonal antibodies to the appropriate level of purity required for their intended application. The purification process significantly impacts the quality and performance of the final product, necessitating an optimized protocol that balances yield, purity, and cost-effectiveness This study aims to optimize the purification process of mAbs using various chromatography columns and buffers in Bio-Rad NGC Chromatography system to achieve the highest yield and purity at the lowest cost. To determine the most effective purification strategy, initially, supernatants were collected from two hybrid cell lines producing mAbs with IgG1 isotypes using hybridoma technology. Subsequently, ammonium sulfate precipitation was performed, followed by testing different chromatography columns, including Bio-Rad UNOsphere SUPrA, Bio-Rad Foresight Nuvia cPrime, and Bio-Rad EconoFit CHT Type II; each paired with their optimal buffers. UNOsphere SUPrA, designed based on affinity to Protein A, efficiently purifies IgG antibodies with high capacity and fast processing times. Foresight Nuvia cPrime uses hydrophobic interaction chromatography (HIC), interacting with hydrophobic regions of proteins to facilitate separation and purification. EconoFit CHT Type II utilizes hydroxyapatite chromatography, relying on interactions with calcium and phosphate groups for protein purification. Moreover, high purity and yield in purifying monoclonal antibodies can be achieved using a combination of different types of chromatography. Purified antibodies were assessed for concentration and purity using spectrophotometric calculation, BCA Assay, enzyme-linked immunosorbent assay (ELISA), silver staining, and Western blot analysis. As a result, it is demonstrated that affinity chromatography is significantly more effective in purifying mAb than other methods.

Keywords: Monoclonal antibodies, Antibody purification, Column chromatography

P180
Designing and Cloning Guide RNA for CRISPR-Cas13b

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Background/Aim: CRISPR/Cas13 expands the CRISPR/Cas9-mediated DNA editing approaches to the RNA editing. In this system, a guide RNA (gRNA) targets a specific region in the RNA of interest and recruits Cas13. gRNA can be designed with little restriction to cover almost the whole transcriptome and various engineered Cas13 enzymes with unique added features can be utilized at the region of interest.

Materials and Methods: Plasmids were obtained from Addgene plasmid repository and their integrities were first confirmed by restriction enzyme digestions. An oligo that is complementary to the region surrounding the start codon of *PATZ1* mRNA was designed and cloned into a gRNA plasmid by using a golden gate reaction. The cloned plasmid was confirmed by Sanger-sequencing.

Results: A 45-nucleotide long sequence that is complementary to *PATZ1* mRNA around the AUG start codon with a mismatched cytosine for the corresponding adenine at the 30th nucleotide from the 3' end of the sequence was designed and cloned into gRNA plasmid.
Conclusion: A gRNA plasmid that targets the start codon of human *PATZ1* mRNA was designed and cloned. When this plasmid is co-transfected into cells with a catalytically inactivated Cas13 fused to an adenosine deaminase encoding plasmid, the adenine nucleotide in the canonical start codon of PATZ1 is expected to be edited to inosine. This change might be functionally important to study the decrease in protein translation or the truncation of N-termini in future studies.

Keywords: CRISPR, Cas13, ADAR, PATZ1, RNA editing

P181
Computational Insights into the Transcriptional Regulation Underlying Cellular Differentiation and Metastasis

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Background/Aim: Transcription factors (TFs) are primary drivers of gene regulation which operate by binding to specific sites on DNA with adequate affinity and stability. Their cooperativity as homodimers or heterodimers are also common for more delicate regulation. Granyhead-like 3 (GRHL3) and Hepatocyte nuclear factor 4 alpha (HNF4α) are TFs that operate as heterodimers to initiate mesenchymal to epithelial transition (MET), a cell-fate alteration process important in development and cancer progression. In addition, NF-κB p50 is a TF playing roles in various physiological processes including inflammation, immunity, cell division, cellular differentiation, and survival. Structural studies suggest for NF-κB p50 a homodimeric mode of cooperativity.

Materials and Methods: We developed structural models of naked- or nucleosomal DNA bound to human GRHL3, as well as models including also HNF4α. In addition, we prepared models of DNA-bound NF-κB p50 as a monomer and as a homodimer. We performed atomistic molecular dynamics (MD) simulations of our models.

Results: We found that GRHL3 and HNF4α cooperate when GRHL3 is bound to its native sequence. Interestingly, the proteins dissociate from one another when this native sequence is mutated. By simulation of DNA-bound NF-κB p50 as a monomer and as a homodimer, we found progressive bending of DNA in the latter—suggestive of initial DNA melting.

Conclusion: We argue that TFs as heterodimers and homodimers have the potential to alter the conformation and the dynamics of DNA, and yet this cooperativity is challenging to characterize experimentally in a spatio-temporal manner. Our findings demonstrate that the DNA sequence is crucial for the cooperativity between GRHL3 and HNF4α to induce MET. Heterodimer formation of GRHL3 and HNF4α and homodimer formation of NF-κB p50 alter the binding capacity of proteins to DNA, consequently altering the DNA dynamics and conformation.

Keywords: transcription factors, cooperativity, molecular dynamics simulations, dna binding, mesenchymal to epithelial transition

P182
Anticancer Activities of Newly Synthesized Metal Compounds in Lung Cancer Cells

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Background/Aim: Lung cancer is the most frequent cancer and the main cause of cancer deaths worldwide. New drugs with low side effects and cost are needed for treatment. These medications should inhibit or stop tumor growth. This is why the produced chemical agents' cytotoxic and migratory effects on the A549 cell line are studied.

Materials and Methods: A549 cells were maintained in DMEM medium with 10% FBS at 37°C and 5% CO2. The chemicals 396-G, 513-G, and 529-G were dissolved in DMSO incubated with the cells at 0 to 100 micromolar concentrations for cytotoxicity (MTT) assay for 48 hours and IC50s were calculated. Additionally, wound healing, colony formation assays were adopted to evaluate anti-cancer activities of the compounds. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to measure apoptotic genes p53 and Bcl2 expression.

Results: For cytotoxicity assay, IC50s of 396-G, 513-G and 519-G were calculated as 2.65 µM, 4.51 µM and 5.02 µM, respectively. In wound closure experiment, the drug 513-G was the best inhibitor of cell motility. At 24h, closure percentages were 31.42%, 20.54% and 39.66% for 396-G, 513-G and 519-G, respectively. At 48h, closure percentages were 32.61%, 31.71% and 40.69%. The qRT-PCR analysis revealed that treatment with medicines 396-G and 513-G resulted in a decrease in the expression of p53 and Bcl2 compared to the control group. Conversely, treatment with drug 519-G led to an increase in p53 and Bcl2 expression.

Conclusion: According to this study 396-G exhibited the highest efficacy and in the wound closure experiment, 513-G was the most efficacious agent in inhibiting cell migration. All of the studied compounds effectively inhibited the development of colonies. Hence, the new compounds have promising activities as potential anticancer drug candidates

Keywords: adenocarcinoma, cytotoxicity, wound healing, colony formation assay, qRT-PCR

P183
AlphaFold Predictions Reveal Polymerized Structures of ZBTB Proteins

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Background/Aim: The members of the ZBTB (Zinc finger and BTB domain containing) protein family, a family of transcription factors encoded by 49 genes in the human genome, take part in multiple roles in the cell such as chromatin structure regulation, stem cell differentiation, cellular oxidative phosphorylation, and the p53 pathway. The BTB (broad-complex, tram-track, and bric-a-brac) domains of these proteins form obligate homodimers. In this study, we investigated the polymerization capabilities of BTB domain homodimers. Recently, our group and others showed that 22 out of the 49 BTB domains which contain a conserved motif including a critical serine residue, homopolymerize and form nuclear condensates when overexpressed. We show that even ZBTB proteins that do not contain this conserved motif such as ZBTB27 can also form condensates when co-expressed with ZBTB9 that contains the motif.

Materials and Methods: We investigated the mechanism of this polymerization using computational methods, specifically AlphaFold 3 and AFsample 2, an implementation of AlphaFold 2 that uses randomly masked multiple sequence alignments, dropout at inference, and increased sampling to increase the diversity of the identified structures. The analysis of the generated structures was done using Python and ChimeraX.

Results: The large-scale sampling generated multiple possible interaction interfaces for ZBTB9-ZBTB27 with varying confidences. The analysis of the generated predictions revealed two possible ZBTB9-ZBTB27 polymerization interactions.

Conclusion: Our predictions indicate that ZBTB9-ZBTB27 polymerization is mediated by a novel interaction interface which correlates with in vitro findings.

Keywords: ZBTB proteins, polymerization, AlphaFold

P184
miR-711 and TLR4 gene expression in amyloid-beta toxicity model of SH-SY5Y cells

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Background and Aim: Neuroinflammation has a significant effect on Alzheimer's Disease (AD), which is characterized by dementia and cognitive dysfunctions. Damage-associated proteins bind to TLR4, activating the NLRP3 inflammasome complex, which causes cytokine release and pyroptosis. As an anti-inflammatory mediator, miR-711 has been shown to bind TLR4 mRNA and affect NLRP3 inflammasome complex in DMD. However, the expression level and regulatory role of miR-711 in amyloid beta oligomer (Aβo) toxicity have not been elucidated. Here, we aimed to reveal the role of miR-711 via its target gene, TLR4, in Aβo toxicity-induced SH-SY5Y cells.

Materials and Methods: The binding potential of miR-711 to TLR4 mRNA was identified *in silico*. SH-SY5Y cells were treated with Aβo for 24-48 hr to induce amyloid toxicity. Following total RNA isolation, the expression levels of miR-711 and TLR4 were determined by qRT-PCR. The results were analyzed by ddCt method, using RNU6 and β-actin as housekeeping genes. $p<0.05$ values were regarded as statistically significant.

Results: *In silico* analysis showed strong binding of miR-711 to TLR4. miR-711 was found to be significantly downregulated (fold change=0.5), and TLR4 was found to be upregulated (fold change =3) in Aβo toxicity-induced SH-SY5Y cells.

Conclusion: We showed for the first time that miR-711 expression is downregulated in Aβo-treated cells, and TLR4 mRNA is upregulated accordingly. Our preliminary results highlight the potential of miR-711 in neuroinflammation. In the future, we aim to assess the role of miR-711 on NLRP3 inflammasome complex formation and pyroptosis.

Keywords: Alzheimers Disease, microRNA, NLRP3, TLR4, Amyloid beta

P185
Assessment of the co-effects of NRF2 Activator and Inhibitor with a Novel Organ-on-Chip Model

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Background/Aim:NRF2 (Nuclear-factor-erythroid2-related-factor-2) is a transcription factor essential for the antioxidant system and involved in various diseases, including cancer. In cancer, NRF2 has a dual role as both a tumor suppressor and a proto-oncogene, complicating therapeutic strategies. Our long term goal is to determine the combined effects of NRF2 activators and inhibitors by developing an organ-on-chip (OOC) that allows for 3D co-culture and local application of inhibitor.

Materials and Methods: An OOC system of multiple channels with access to a common continuous interface was designed. OOCs were fabricated using soft lithography and molds fabricated with 3D printing. Local diffusion was examined theoretically by VCell simulations and experimentally using Alexa488 and fluorescence microscopy. MDA-MB-231, MCF7, SKBR3 and MCF10A cells were used to represent different breast cancer types and normal mammary epithelial cells, respectively. Cell viability was determined using Calcein AM/PI as well as Alamar Blue staining followed by fluorescence microscopy.

Results: Local diffusion results from VCell simulations and fluorescence microscopy were in agreement indicating that local inhibitor application is possible in the OOC. Cell seeding densities of $2x10^6$, $1x10^6$, and $5x10^5$ cells/ml were tested. Highest cell viability as determined by Alamar Blue fluorescence intensity measurements was observed for all cell lines at $5x10^5$ cells/ml. Cell viability results were confirmed using Calcein AM/PI staining imaged using fluorescence microscopy and quantified via image analysis. A patent application for the OOC has been filed.

Conclusion: An OOC system that allows local maxima of soluble molecules was optimized for 3D co-culture of cancer and normal cells. Future studies will determine the co-effects of NRF2 inhibitor and activator in the presence and absence of drug.

Keywords: NRF2, cancer, organ-on-chip

P186
Functional Investigations of Pathogenic Variations in CCN6, Progressive Pseudorheumatoid Dysplasia Disease Gene, in Chondrocyte Cell Lines

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Aim / Background: Progressive pseudorheumatoid dysplasia (PPD) is a rare skeletal and joint disease of the children and inherited as an autosomal recessive mode. Causative gene for PPD is Cellular Communication Network Factor 6 (*CCN6*), is associated with growth factors in connective tissue and encodes a protein called WNT1-inducible signaling pathway protein 3 (WISP3). The pathophysiology of PPD is believed to be influenced by dysregulation the cartilage homeostasis through *CCN6* pathogenic variations. However, the exact molecular mechanism is not fully understood.
The aim of the study was to gain insights into the pathogenesis of the disease through cell of culture models. Commonly observed pathogenic variants of our PPD population were genome edited in primary human chondrocytes, and expressions of cartilage homeostasis genes were investigated.

Materials and Methods: The c.327C>A and c.342T>G substitutions were created in human WISP3 cDNA clones by site-directed mutagenesis and confirmed by Sanger sequencing and transfected into human chondrocyte cells to obtain over-expression of *CCN6 variants*. After RNA isolation, cDNA synthesis was performed and the expressions of *FGF-19, SOX9, IL1B, NFR2, COL2A1, MMP1,* and *COX-IV* were analyzed by quantitative PCR. To assess alterations in WISP3 and its interacting proteins within the extracellular matrix, cell adhesion assay was conducted.

Results: *IL1B* expression was significantly higher in overexpressed c.327C>A variant compared to both c.342T>G and the wild type in qPCR analysis. Also, *COXIV* expression decreased in the c.327C>A variant,revealing this variant may cause dysfunction in mitochondria. Reduced *SOX9* expression was seen in both variants, indicating that variations in *CCN6* leads to dysregulation of cartilage homeostasis, regardless of whether they are nonsense or not. In addition the c.327C>A variant construct largely lost its interaction with gelatin and collagen in attachment assay.

Conclusion: Intracellular localization of variants and their effects on reactive oxygen levels are ongoing.

Keywords: chondrocytes, WISP3, CCN6, Progressive Pseudorheumatoid Dysplasia, Genome editing

P187
Functional Characterization of Intellectual Disability Associated RNFT2 Gene

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Background / Aim: RNFT2 (Ring Finger Protein, Transmembrane 2) is an E3 ubiquitin ligase that facilitates substrate recognition and subsequent degradation by regulating ubiquitin attachment. Previously, we investigated the RNFT2 ortholog in flies, DmelCG13605, finding that knockout mutants exhibited defects in axonal pathfinding and mushroom body neuron elongation, essential for cognitive functions like learning and memory (Kahraman, 2022). To explore the association between the RNFT2^{C384R} mutation, found in individuals with intellectual disability (ID), with the ID phenotype, we aim to uncover the cellular mechanism of RNFT2.
Methods / Materials: To study the subcellular localization of wild-type and mutant RNFT2, the cDNA was cloned into the pLex307 vector using Gateway cloning. HeLa cells were stably transfected, and the localization of V5-tagged RNFT2 was addressed using different organelle markers. The localization of V5-tagged RNFT2 was analyzed with an RNFT2 antibody. To evaluate protein stability, a cycloheximide (CHX) chase assay was performed.
Results : Co-localization studies in stably transfected HeLa cells revealed that V5-tagged RNFT2 predominantly localizes to the Golgi apparatus. The RNFT2^{C384R} mutation did not alter this localization. We confirmed that endogenous RNFT2 localization matches that of the V5-tagged protein. The CHX chase assay indicated that RNFT2^{C384R} is less stable than wild-type RNFT2, showing faster degradation.
Conclusion: The RNFT2^{C384R} mutation does not change the protein's subcellular localization but significantly reduces its stability, suggesting a potential role in the pathogenesis of intellectual disability. This mutation may lead to disruptions in ubiquitin-mediated processes in neurons. Further research involving the identification of the interactome, CRISPR mutants, and analysis of the ERAD pathway will help to detail how this mutation affects neuronal function and contributes to the observed phenotypes.
This work was partially supported by TÜBİTAK Grant No. 119Z309 and TÜSEB Grant No. 32759.

Keywords: Intellectual disability, ubiquitination, ERAD, fly model

P188
Roles of miR-155 in the Colorectal Cancer Tumor Microenvironment During Immune Checkpoint Inhibition

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Background: Colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide and the fourth leading cause of cancer-related deaths. Increasing rates of CRC and poor survival rates the need for effective treatment options. Immune checkpoint inhibition (ICI) has shown promising results, but it isn't effective for all patients, and the reasons for its varying efficacy remain unclear. Research is ongoing in several directions to address this issue, with efforts focused on understanding the role of microRNAs (miRNAs) in tumor immunity.miRNAs are small non-coding RNAs that can control hundreds of target genes. miR-155 has more recently emerged as a regulator of tumor immunity due to its effect on anti-tumor activity by T cells, but the pathways it regulates in the context of immunotherapy are not fully understood.

Aim: This study aims to investigate the roles of miR-155 in T cells within the tumor microenvironment (TME) during ICI treatment in preclinical models of CRC.

Methods and Results: Immune cells expressing CD45 were FACS-sorted from MC-38 tumors grown in WT or miR-155 T cell knockout (TCKO) in the presence or absence of ICI.All subsequent analyses were performed using the Seurat R package, including normalization, integration, dimensionality reduction, and clustering. Fourteen distinct cell clusters were identified including multiple populations of myeloid and lymphoid lineages. The most dramatic change was in the frequency of cells from the CD8 T cell cluster, approximately 5-fold lower in miR-155 TCKO immune cells. Differential gene expression analysis comparing WT and miR-155KO CD8 T cells revealed enrichment of effector-related genes in miR-155 competent CD8 T cells.Cluster-wise Gene Set Enrichment Analysis(GSEA) was performed between select pairs of samples. ICI treatment in the presence of miR-155 exhibited a high enrichment score for genes associated with IFNy response and allograft rejection pathways.

Conclusion : Within the TME, miR-155 expression in CD8 T cells appears to be crucial for anti-tumor immune responses during ICI.

Keywords: Immune Checkpoint Inhibition, CD8 T cell, MicroRNA-155

P189
Characterization of CG13531 Knockout Mutants in Drosophila melanogaster as a Model for SPG11-Associated Motor Neuron Neuropathies

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Background/Aim Motor neuron neuropathies are characterized by a spectrum of symptoms and pathological mechanisms, such as motor neuron degeneration, speech and movement difficulties, and muscle wasting. The *spastic paraplegia gene 11* (*SPG11*) is associated with several neuropathies, including hereditary spastic paraplegia, amyotrophic lateral sclerosis, and Charcot-Marie-Tooth disease. It codes for a transmembrane and potential scaffold protein named spatacsin, known to play a role in axon extension, axonal transport, and lysosome and autophagosome organization. *CG13531* is the predicted ortholog of *SPG11* in *Drosophila*; however, its functional similarity to SPG11 has not yet been demonstrated. This study aims to characterize the knockout mutants of *CG13531* and generate a fly model of *SPG11*-dependent symptoms. To that end, we investigated the morphological and behavioral phenotypes occurring in the absence of *CG13531*.

Materials and Methods: We used *CG13531CRIMIC* mutants generated by the Bellen lab. The neuromuscular junctions (NMJs) of the mutant larvae were subjected to immunohistochemistry and morphological analysis to assess synaptic development and functionality. The locomotor abilities of young and old flies were measured using startle-induced negative geotaxis assay. The survival and eclosion rate of the mutants was investigated to characterize the lethality and severity of the mutation.

Results: *CG13531* knockout mutants exhibit an age-dependent decline in the climbing ability, an increase in the number of boutons, and an increase in axonal branch number at the NMJ. Additionally, the *CG13531* knockout mutation leads to a reduced lifespan in flies, although their emergence as adults is not affected by the mutation.

Conclusion: *CG13531* is important for proper NMJ development, and its absence leads to underdeveloped synapses. Loss of *CG13531* also causes locomotor defects that manifest later in life. Its role in cellular pathways and neurodegeneration will be thoroughly investigated to create a well-defined model.
This project was supported by BAP project no. 19780 to AÇF.

Keywords: motor neuron degeneration, Drosophila melanogaster, ALS, HSP, SPG11

P190
Comparison of the Genetic Determinants of Colorectal Cancer Metastasis Using Publicly Available Datasets

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Background/Aim: Colorectal cancer is one of the most common leading causes of cancer related deaths due to its metastatic ability which accounts for nearly 95% of cancer related deaths. Gaining metastatic characteristics is highy dependent on the genetic background of primary tumours. Metastasis is a complex process that involves genetic and molecular changes, making the genetic background of primary tumors a critical area of study. Understanding these genetic alterations can provide insights into the mechanisms driving metastasis and potentially lead to the development of novel therapies.

Materials and Methods: In this study, we compared the genomic backgrounds of microsatellite instable (MSI) CRC tumours: early primary (60), primary (19) and metastatic (10) using a publicly available targeted sequencing dataset (PRJEB23844) with an ultimate aim to understand genetic determinants for metastasis process. Mutation percentages of cancer related genes in these groups were calculated and candidate genes were identified.

Results: Results revealed a gradual increase in the mutation frequencies of the *DIS3, DOT1L, MSH2, CIC, ARID1B* and *AXIN2* genes from early primary through primary and metastatic samples. Also, gene enrichment analysis between the same groups revealed that distinct cancer related pathways were enriched. Furthermore, the maintanance of DNA repeat elements was identifed as potential cancer related pathways.

Conclusion: These findings suggest the importance of genomic background of primary tumour in understanding the process of colorectal cancer metastasis and show the involvement of specific genes and pathways in this process which could in turn potentially serve as targets for therapeutic intervention.

Keywords: metastasis, colorectal cancer, primary tumor, candidate pathways, candidate genes

P191
Genome related features of introns and exons reveal new properties and pareto fronts

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Aim: Genome annotations reveal vast amount information, as well as genome related features. However, thinking that their global features are under-investigated, we aimed to look into such features.

Methods: We looked at the genome related features of eukaryotes, grouped as primates, rodents, birds, fishes, insects, and plants.

Results: Features related to introns and exons reveal distinct assets. Genes' mean lengths are positively correlated with the collective data of counts of exons in coding transcripts and both the mean lengths and counts of introns in coding transcripts. Mean lengths of introns in non-coding transcripts are also positively correlated and correlations with the counts-data are excluding the data of plants and fishes. These are valid for the median lengths as well. In addition, certain feature comparisons reveal separation of primates, rodents, birds, fishes, insects, and plants. We also observed pareto fronts in the cumulative data with the max length values of genes and introns in coding transcripts, as well as introns in coding and non-coding transcripts.

Conclusion: Genome related features of eukaryotes reveal distinctive and unique information

Keywords: Genome annotation, length feature, count feature, eukaryotes

P192
Investigation of the roles of KDM6A and KDM6B histone demethylases in isocitrate dehydrogenase (IDH) mutant glioma

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Background/Aim: Gliomas, which account for 80% of malignant brain tumors, are notably aggressive. Most IDH-mutant gliomas feature a heterozygous point mutation in IDH, leading to 2-hydroxyglutarate accumulation. This accumulation inhibits DNA and histone demethylases, resulting in a hypermethylated phenotype. We aimed to examine the epigenetic vulnerabilities of IDH1-mutant gliomas. A chemical screen of chromatin modifiers previously identified GSK-J4, an inhibitor of histone demethylases KDM6A and KDM6B, selectively effective against IDH-mutant cells. In this study, we aimed to establish a genetic approach to phenocopy the effects of chemical inhibition of KDM6A/6B.

Materials and Methods: We employed CRISPR/Cas9-based gene ablation in primary IDH1-mutant glioma cells, MGG152 and TS603. Standard molecular cloning, qRT-PCR, western blot, Annexin V staining, oxidative stress assay, and cell titer glo assay were used for cell-based assays, and RNA sequencing was employed to interrogate transcriptomic changes upon KDM6A/6B loss.
Results: We established IDH-mutant primary lines with reduced KDM6A/6B expression. Double knockout displayed reduced growth rates, morphological differences, and increased H3K27me3 levels. Apoptosis assays detected no significant increase in apoptosis-related phenotypes. RNA sequencing analysis revealed upregulation of transforming growth factor beta (TGF-β), its targets, and senescence-related genes. To test the link between KDM6A/6B-regulated TGF-β and reactive oxygen species (ROS), we compared ROS levels in KDM6A/6B-depleted cells and controls. To assess the relation between ROS and senescence, we performed β-galactosidase staining. As a more in vivo-compatible cell line, TS603 cells resulted in tumor formation with histological features of gliomas. We tested the tumor formation ability of KDM6A/KDM6B-depleted TS603 cells in mice.

Conclusion: Despite their diverse roles in various tumors, the functions of KDM6A and KDM6B in IDH-mutant gliomas remain underexplored. Our findings shed light on the significant roles of KDM6A and KDM6B in IDH-mutant gliomas and suggest new therapeutic strategies involving epigenetic interventions.

Keywords: Glioma, epigenetics, IDH mutation, cancer

P193
A Novel Bioactive Chimeric Antibody for HER2 Targeted Therapy

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Background/Aim: HER2 (human epidermal growth factor receptor 2) is responsible for proliferation, survival, angiogenesis and mainly overexpressed in breast, gastric, lung and colon cancer. Trastuzumab and pertuzumab are two humanized monoclonal antibodies recognizing different epitopes on HER2. Despite widespread usage, their clinical efficacy remains limited in patients who develop resistance over time. In this work, we aimed to develop novel antibody for use in HER2-positive cancers that would offer an alternative or novel dual therapy.

Materials and Methods: We previously reported on the production of an anti-HER2 mouse antibody with hybridoma technology. In this study, we sequenced and identified cDNA of variable regions. To produce mouse-human chimeric antibody we designed two expression vectors encoding heavy and light chains with mouse variable and human constant regions. Plasmids were stably expressed in Hek293T cells to produce chimeric antibody. Antibody secreted into medium was purified by affinity chromatography. The efficiency of the antibody was tested using flow cytometry and ELISA. The anti-growth effect of the chimeric antibody both alone and in combination with trastuzumab and pertuzumab, was tested in HER2 overexpressing, NCI-N87 cells.

Results: Chimeric antibody recognized both recombinant and native HER2 receptor. Antibody was able to bind HER2 in the presence of trastuzumab or pertuzumab. The antibody showed significant anti-growth effect in NCI-N87 cells. When the antibody was combined with trastuzumab, it showed in vitro synergistic effect which was superior to the anti-growth impact of the trastuzumab and pertuzumab combination. However, when used in combination with pertuzumab, there was no synergy.

Conclusion: Our chimeric antibody has significant anti-growth effect in HER2-expressing cells. The antibody binds to HER2 even in the presence of trastuzumab and pertuzumab. This makes it a suitable candidate for use in dual targeted therapies. Moreover, it is promising that when used in combination with trastuzumab, it outperforms the current dual regimen of trastuzumab and pertuzumab against HER2 positive cancers.

Keywords: HER2, targeted therapy, monoclonal antibody, chimeric antibody

P194
Induction of Rosmarinic Acid Production in Lavandin Cell Suspension Culture via Salicylic Acid, Methyl Jasmonate and Proline

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Background/Aim:
The secondary metabolites of lavandin, in particular rosmarinic acid (RA), are recognized as significant bioactive compounds in pharmaceutical, cosmetic and aromatherapy industries. RA is a polyphenolic secondary metabolite with anti-inflammatory, antioxidant, anti-carcinogenic and anti-aging effects. The production of RA in cell suspension cultures can be enhanced with elicitors by inducing stress responses in plant cells. Methyl jasmonate (MeJA) is a crucial abiotic elicitor for plant stress adaptation while salicylic acid (SA) regulates the synthesis of antioxidant phenolic compounds including RA. Proline is a well-known stress indicator that can increase secondary metabolite production by mimicking stress conditions. The objective of this study is to enhance RA production in lavandin cell suspension culture and assess the impact of different cell lines on RA production, both intracellularly and extracellularly.

Materials and Methods: Lavandin cell suspension cultures were treated with SA (50 µM, 100 µM), proline (0.5 mM, 5 mM), or MeJA (5 µM, 10 µM), with a control group containing no elicitors. The experiment was conducted in two trials where multiple cell lines or a single cell line is utilized. Both intracellular and extracellular RA production was determined by UV-VIS spectrophotometry.

Results: In the first trial, using multiple cell lines, application of 100 µM SA showed the highest increase in intracellular RA levels, followed by 10 µM MeJA treatment. In the second trial, using a single cell line, 10 µM MeJA application led to an increase in extracellular RA production. Besides, 10 µM MeJA (
$p \leq 0.05$) treatment yielded the highest total RA production among all treatments.

Conclusion: This study highlights the potential of SA and MeJA as elicitors to enhance RA production in lavandin cell cultures. Though RA in lavandin cells is produced intracellularly, by the use of elicitation, not only RA production is enhanced but also extracellular production is triggered.

Keywords: Rosmarinic acid, Lavandin, Plant cell suspension culture, Elisitation

P195
Identification of the Bi-potency of Liver Cell Types Under Fibrosis

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Background and Aim: Liver is populated by a variety of cell types ie. cholangiocytes, hepatocytes and has been known to be capable of regenerating its cell population upon damage such as fibrosis. Upon persistent liver damage, coupled with the impairment of hepatocyte proliferation, cholangiocyte population shows a leniency to acquire an expression profile resembling to hepatocytes. However, this leniency has not yet been demonstrated without the external regulation of the pathways to push cells to hepatic fate in an organoid system. Here, we provide evidence that ductal organoids derived from TAA treated mice models carries a transcriptional reprogramming that allows these cells to become more prone to acquire a hepatic like phenotype without the need of external modulation but only the transcriptional reprogramming of the damaged cells itself.

Material and Methods: Using organoid models, we demonstrate a difference at the expression profiles and organoid forming capacity of organoids generated from TAA treated animals and control animals. In this study, by the construction of liver assembloids using ductal and hepatocyte like organoids that are derived from same origin a dynamic model that mimics the complexity of liver physiology in a more accurate manner is represented.

Results and Future Perspectives: Here, we present a mechanism in which upon chronic damage liver progenitor cell population pool is contributed by cholangiocytes that have a leniency towards hepatic cell fate; and a model to mimic the interaction of these two cell types in complexity. This study will contribute to understanding and modeling liver fibrosis and characterize the potency of liver cell types in a more detailed manner. Through our findings it is evident that Liver Assembloids form fused structures that resembles the alignment of native tissue structure. Future studies will be focusing on the additional aspects such as the presence of zonation markers which will further emphasize the capacity of liver assembloids mimicking liver tissue.

Keywords: Organoid, Assembloid, Fibrosis Model, Regeneration, Animal Model

P196
Elucidating the Role of Novel ZAD Protein, CKIIα-i1, in Drosophila melanogaster Nervous System Development and Neuroblast Differentiation

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Background/Aim: The development of the nervous system in Drosophila melanogaster originates from pluripotent cells called neuroblasts and is regulated by a transcription factor class called Hox genes. While Hox genes initially control the main cellular functions, other transcription factor (TF) classes, such as ZAD (Zinc-finger-associated domain) proteins, also play crucial roles. The ZAD domain is a protein-protein interaction site found almost exclusively in insects. CKIIalpha-i1, a poorly studied gene, contains one ZAD domain and four different C2H2 domains. It has been shown to interact with proteins involved in nervous system development. This study investigates the role of CKIIalpha-i1 in nervous system development and neuroblast differentiation.

Methods and Materials: A Gal4 driver with the upstream region of CKIIalpha-i1 was generated for this study. The expression pattern was elucidated using this Gal4 line with reporters, a BAC line producing CKIIalpha-i1:GFP fusion protein, and RNA in situ hybridization technique. Mutant lines were generated using the CRISPR/Cas9 system for knock-out studies. CKIIalpha-i1 with a V5 tag was expressed in Schneider-2 cells.

Results: CKIIalpha-i1 is expressed during early embryonic stages, exclusively in neuroblasts and neuroectodermal tissue. In the adult stage, it is found in circadian rhythm clock neurons and photoreceptor cells. Mutants exhibit abnormalities in the α3 lobe of mushroom bodies. CKIIalpha-i1 localizes to the nucleus; however, the protein product of the gene is smaller than the predicted size, suggesting cleavage at the N terminus and near the ZAD domain.

Conclusion: CKIIalpha-i1 is expressed in the nervous system and neuroblasts of Drosophila. It localizes to the nucleus and is possibly cleaved from the ZAD domain, indicating a potential novel control mechanism for the ZAD protein family. Further investigation into the cleavage site and its implications is planned. The study will also identify DNA binding targets and protein interaction partners to understand CKIIalpha-i1's function.
This work was partially supported by TÜBİTAK grant no. 119Z309.

Keywords: Drosophila melanogaster, Nervous System Development, Transcription Factors, Neuroblast

P197
Lab-on-a-chip device (LOC) for Determining Drug Dose Response

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Background: It is important to determine the dose that is effective and at the same time has minimum side effects. It is also important to determine toxic doses as well as doses that do not result in an effect. In classical 2D cell culture, serial dilutions are often used to test different doses of drugs. However, this approach is prone to pipetting errors. A more critical disadvantage is the lack of a physiologically relevant microenvironment. One solution is to use lab-on-a-chip (LOC) devices that can generate gradients of drugs. Such current devices are complex in structure, require flow and are difficult to fabricate. Here we provide a novel, easy to fabricate LOC that can determine cell viability to different drug doses in a single LOC, in the absence of flow, in physiologically relevant conditions, designated as the DRCHIP.

Materials and Methods: DRCHIP was fabricated using 3D printing. Alexa488 was used to determine diffusion. MDA-MB-231 breast cancers cells were exposed to paclitaxel in DRCHIP. Viability was determined using Calcein-AM and Alamar Blue assays.

Results: We investigated the diffusion profile of small molecule drugs in DRCHIP using Alexa488 as an avatar with VCell simulations and fluorescence microscopy imaging, both of which were in agreement. We then determined the viability of MDA-MB-231 breast cancers cells exposed simultaneously to different doses of paclitaxel in the DRCHIP using Calcein-AM and Alamar Blue. As expected, regions in the DRCHIP with higher dose of paclitaxel exhibited lower cell viability with an IC50 of ~70 mg/kg.

Conclusion: Our results show that the DRCHIP can be used to determine drug dose response in a physiologically relevant microenvironment.

Keywords: lab-on-chip, cancer, drug response

P198
Investigating the Phenotypic Variations In Isogenic Drosophila Lines

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Background/Aim: Drosophila transcription factor Bicoid (Bcd) is a novel factor in insects that is essential for the embryo development. Bcd recognizes specific target genes through its DNA binding Homeodomain (HD) and regulates hundreds of genes along the AP axis involved in segmentation. Previous research identified key sequence changes in Bcd HD region with evolutionary consequences. Here we focus on two mutant strains, in which we observed increased phenotypic variations such as severe head and abdominal defects. To identify the causes of the phenotypic variations in these isogenic lines we conducted perturbation experiments. We also wondered if survivors have any non-genetic (or genetic) advantages by selective breeding of survivors.

Materials and Methods: We used transgenic D.melanogaster control (BcdHD, AncBcd HD) mutant lines (nt-H1-h2-RH, NT-h1-h2-RH). Mutant lines were generated by substituting HD subdomains between nonfunctional AncZB (nt-h1-h2-rh) and functional AncBcd HDs (NT-H1-H2-RH). Embryos from homozygot mothers were analyzed for phenotypic defects. Temperature effects were tested by incubating strains at 25°C and 22°C. Phenotypic variations and survival data were analyzed to assess the impact of genetic and environmental factors. For inheritance and evolution of variable phenotype (survival), we selected survivors after each generation and crossed them with each other to investiage the phenotypic and Molecular changes.

Results: Mutant Drosophila melanogaster lines (nt-H1-h2-RH and NT-h1-h2-RH) displayed significant phenotypic defects, with survival rates lower than 4%. At 22°C, survival increased slightly in nt-H1-h2-RH from 3.2 to 3.6%. Selective breeding studies indicated improved phenotypic variation in NT-h1-h2-RH, with survival rates rising from 1% to 13% in F2, suggesting variations are inheritable. In contrast, nt-H1-h2-RH showed no significant survival change suggesting variations might be stochastic.

Conclusion: Study highlights the role of non-genetic and stochastic changes in developmental regulation and evolution. In the future we will investigate, mechanisms of potential non genetic inheritance as well as mechanisms of stochasticity by further molecular and perturbation experiments.

Keywords: Bicoid, Transcription, Drosophila, Development

P199
Astrocyte Activation and Aryl Hydrocarbon Receptor Signaling: Implications in Neurodegeneration Research

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Background/Aim: Astrocytes provide essential metabolic support to neurons by regulating the supply of energy substrates, maintaining ion balance, and modulating neurotransmitter levels; however, under conditions of metabolic stress, their dysfunction can contribute to neurodegeneration by shifting from a neuroprotective to a neurotoxic phenotype. This study investigates the effects of stress factors applied on astrocytes and their relationship with Aryl hydrocarbon Receptor (AhR) signaling, which is known to mediate cellular responses to environmental toxins and stressors.

Methods: Immortalized human astrocytes were treated for 24 hours with various agents and doses:10,100 ng/mL TNF-α (Tumor Necrosis Factor-alpha), 100 ng/mL LPS (Lipopolysaccharide), 2.5,10 ng/mL TGF-β1 (Transforming Growth Factor-beta-1), 100,1000 nM FICZ; AhR-agonist (6-Formylindolo[3,2-b]carbazole), 1.5 µM SR1; AhR-antagonist (StemRegenin-1). Metabolome analyses of the supernatants were performed using gas chromatography-mass spectrometer (GC-MS). RT-qPCR was used to analyze 20 neuroprotective and neurotoxic astrocyte- and AhR-specific genes.

Results: Higher ornithine levels were noted in the TNF-α, LPS, SR1, and low-dose TGF-β1 groups, while lower levels were found in the high-dose TGF-β1 group. Increased expression of inflammatory genes was observed in the TNF-α and LPS groups, with no change in the TGF-β1 group. Significant changes in both neurotoxic and neuroprotective-specific genes were noted in the FICZ and SR1 groups.

Conclusion: Metabolome analysis revealed higher ornithine levels and lower energy metabolites in the TNF-α, LPS, SR1, and low-dose TGF-β1 groups, proposing ornithine as a potential therapeutic target in neurodegeneration. No significant changes were observed in neurotoxic and neuroprotective gene expression in the TNF-α, LPS, and TGF-β1 groups. However, the FICZ and SR1 groups showed significant changes in genes, indicating that there may be an intermediate phase in astrocytes. These findings suggest a role for AhR signaling in neurodegeneration. However, further research is warranted to elucidate the role of astrocytes in neurodegeneration.
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Keywords: Astrocyte, Neurodegeneration, neurotoxicneuroprotective-phenotypes, AhR-signaling

P200
In silico Characterization of a Rare Disease that Affects the Dynamics and Function of Linker Histone H1

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Aim: The eukaryotic genome is wrapped in nucleosomes to form chromatin. Nucleosomes contain 147 bp of DNA wrapped ~1.65 times around a histone octamer comprising two copies each of the core histones H2A, H2B, H3, and H4. In higher eukaryotes, a fifth histone, known as the linker histone H1, binds the nucleosome and the linker DNA between nucleosomes to facilitate the folding of the chromatin fiber into a higher-order three-dimensional structure. In late 2019, germline frameshift mutations involving one of the H1 subtypes, H1.4, were causally linked to an as-yet poorly defined syndrome called the Rahman syndrome (RS). In affected individuals, characteristic phenotypes include intellectual disability and skeletal and cardiac anomalies. We hypothesize that the chromatin domains harboring the RS-H1.4 could exhibit a less condensed and differently organized structure, which could disrupt the delicate architecture and function of chromatin.

Material and Methods: To understand how the RS mutant H1.4 affects nucleosome dynamics, we performed atomistic molecular simulations of the WT (wild-type) and the RS-H1.4 bound nucleosome. Among all RS patients, we have simulated the most affected RS-H1.4 from "patient#1".

Results: Indeed, we found that the H1.4 of patient#1 could not only not fold the linker DNA strands but was destabilizing them in comparison to the WT as well as a "tailless" mutant that lacks the CTD.

Conclusion: In conclusion, the findings of this study will not only give us information about the RS but also provide critical findings about the 3D chromatin architecture mechanism. The combination of these studies is expected not only to shed light on the mechanism of RS but could also be used as an example of a "workflow" for rare disease studies.

Keywords: Molecular dynamics, Rare disease, Chromatin, Epigenetics, Linker histone

P201
Unveiling the Role of PSMB10 Gene Splice Variant in Dominantly Inherited Auto-inflammatory Disease: Insights into Autophagy Interaction and Clinical Im

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AIM: The autophagy-lysosome pathway (ALP) and the ubiquitin-proteasome system (UPS) are two major proteolytic processes responsible for the degradation of misfolded and damaged proteins. In this study, we investigated autophagic flux in the peripheral blood mononuclear cells (PBMCs) of a family suffering from undiagnosed systemic autoinflammatory disease (SAID) with a variant of unknown significance (VUS) in the
PSMB10 (rs201451622, NM_002801:exon1:c.56+1G>A) gene. The *PSMB10* gene encodes the beta 2 subunit of the 20S proteasome, and this variant could lead to a splicing error, based on splicing *in silico* analysis. Our objective was to assess both gene and protein expression related to autophagy biomarkers in PBMC samples from affected and healthy individuals.

METHODS: PBMCs were isolated from blood samples collected from affected and healthy individuals. PBMCs were cultured in RPMI 1640 medium and exposed to Bafilomycin A1 (100 nM) to inhibit the autophagy flux. LC3B expression level was determined using Western blotting. *ULK1*, and *ATG14* expression levels were evaluated. Statistical significance of differences was calculated by one-way ANOVA.

RESULTS: Higher autophagic flux observed in affected family members, carrying *PSMB10* variant, compared to unaffected members. In P1, P2, and P4 patients, a 0.2-fold in *ULK1* gene expression and a 0.1-fold in *ATG14* gene expression decrease were observed compared to healthy controls. Similarly, 0.3 and 0.4 fold change (LC3BII/LC3BI) decrease were observed in P2-P3-P4 compared to controls. Increased LC3B-II expression in patients confirmed that high autophagic flux was suppressed.

CONCLUSION: Patients carrying the PSMB10 variant demonstrated higher autophagic flux, indicating a potential role of this variant in SAIDs. This research enhances our understanding of the autophagy pathway and provides preliminary data for treatment process of the patients. Future studies will examine the localization and properties of autophagosomes and determine the relationship between abnormal autophagy and the novel *PSMB10* variant.

Keywords: PSMB10, Autophagy, PBMC, SAID

P202
Investigation into the Role of Septin Filaments in the Formation and Maintenance of Neuromuscular Junctions in Drosophila melanogaster

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Background/Aim: The Septin protein family, the fourth component of the cytoskeleton, consists of GTP-binding proteins that form higher-order filaments. Septins play a role in various cellular processes, such as maintaining cell shape, cytokinesis, and endomembrane trafficking. However, their role in synapse formation still requires better understanding. This research investigates the impact of Septins on the structure of neuromuscular junctions (NMJs). The study examines how Sep2 and Sep5 contribute to the development of larval NMJs. These proteins have redundant functions and serve as the core subunit of Septin hexamers.

Material/Methods: A double knock-out fly line was generated with deletions in Sep2 and Sep5. GFP-tagged Sep2 under the control of its endogenous promoter was used for expression analysis. Following immunostaining, morphometric analysis and quantification of NMJs were conducted. The recycling of synaptic vesicle pools was studied by FM 1–43 labeling.

Results: The Sep2 protein was detected throughout the neuromuscular system of flies, including myocytes and motor neurons. When both Sep2 and Sep5 were mutated, lethality was observed at the beginning of the prepupal stage, and neuromuscular domains were mislocalized. Abnormal localization of synaptic proteins was observed when the presynaptic and postsynaptic membranes were labeled with Synapsin (Syn) and disc large-1 (Dlg-1), respectively. Moreover, double mutant larvae displayed disrupted endocytosis in the presynaptic area, resulting in the abnormal localization of rapid recycling vesicles. The mutation also impacted the localization of glutamate receptors (GluRA) in the postsynaptic membrane.

Conclusion: In conclusion, this study highlights the crucial role of Septins in neuromuscular development, synaptic protein localization, and regulation of endomembrane trafficking. Further investigations, including whole-body and tissue-specific overexpression of Sep2, are underway to rescue the lethality and maldevelopment of synapses.

This work was partially supported by the TEKFEN Foundation.

Keywords: Cytoskeleton, Drosophila, Septin filament, Neuromuscular junction, Membrane trafficking

P203
Functional Analysis of MSH2 A733T Variant of Unknown Significant Mutation Effect on Human MutS Homolog 2 (hMSH2) Protein

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Background: Lynch Syndrome is a genetic disease that increases the risk of various cancers, including colorectal cancer, due to loss-of-function mutations in the DNA mismatch repair (MMR) genes; MLH1, MSH2, PMS2, and MSH6. MSH2 and MSH6 constitute MutSα complex which recognizes and repairs DNA mismatches through binding MutLα complex. When this repair process is disrupted, mutations accumulate by increasing the cancer risk. Our previous studies that screened Turkish population for putative variations on these genes showed multiple variants of uncertain significance (VUS) on MSH2/6 genes. In this study, we aim to characterize these VUSs and understand their functional causes at the protein level.

Materials and methods: We determined the VUSs for functional studies from NGS data of 371 colorectal cancer patients. We initiated our experiments by checking the transcript levels of MSH6 and MSH2 genes with mutants using q-PCR. After transient transfection to LoVo cells, which lacks MSH2 expression, protein levels were studied by treating the cells with 10 μM MG-132, proteasome inhibitor, to determine the effect of mutation on the translation levels of these proteins. *In vitro* binding experiment was performed with MSH2 and MSH6.

Results: Our results showed that the transcriptional expression of MSH6 increased proportionally with the expression of MSH2 WT and mutant. MSH2 also increased the MSH6 expression at protein level. *In vitro* binding experiments showed that MSH2 A733T did not affect the MutSα complex formation.

Conclusion: We concluded that MSH2 A733T mutation did not disrupt the complex formation. There was a clear correlation between the levels of MSH2 and MSH6 expression. Further studies were planned/are necessary to understand how this regulation occurs within the cell.

This study was supported by the Scientific and Technological Research Council of Turkey (TUBITAK), Project Number: 318S127 and Scientific Research Projects Coordination Unit (BAP) of ITU, Project Number: 45858

Keywords: Lynch Syndrome, VUS, MMR, MSH2, MSH6

P204
Recombinant Production, Purification and Characterization of Bioactive Peptides

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Background/Aim: Bioactive peptides (BAPs) are peptides exhibiting physiological effects on living organisms. There are many BAPs with proven antimicrobial, antiviral, antihypertensive, antioxidant, antidiabetic, anticancer, anti-obesity, opioid, immune regulator and cholesterol-reducing properties. Production of bioactive peptides from natural proteins are generally challenging due to low yields and complex purification processes. This study aims to produce selected BAPs using an *E. coli* recombinant expression system to establish a platform for efficient BAP production. The selected peptides , designated peptide-1 and peptide-2, have antimicrobial and ACE inhibitory activities, respectively (Montone et al., 2018; Hu et al., 2019).

Materials and Methods: The genes encoding the peptides were inserted into the pET28a vector fused with N-terminal 6His-SUMO. Recombinant proteins were purified via affinity chromatography and anion exchange chromatography. N-terminal fusions were cleaved using ULP1 protease to yield pure peptides. SEC-SAXS data was collected at P12 BioSAXS beamline at PETRAIII Synchrotron.

Results: 6His-SUMO-peptides were purified as verified by electrophoresis and were characterized using X-ray small angle solution scattering. Comparing data from SUMO fusion and SUMO-peptide-1 revealed that peptide 1 induces dimerization in SUMO fusion, with SUMO-peptide-1 existing in solution as both dimers and monomers. SAXS data for 6His-SUMO-peptide 2 showed increased Rg value and molecular weight compared to SUMO alone, indicating an elongated flexible structure for peptide-2. The bioactivities of the peptides will also be measured to verify functional peptide production.

Conclusion: In this study, we used a strategy to obtain peptides with known sequences by producing them in recombinant systems, thereby facilitating and standardizing purification processes. This approach can be applied to various peptides for food or pharmaceutical applications.

Keywords: recombinant peptide, bioactive peptides, characterization

P205
Glycyrrhizic Acid Showed Ameliorative Effects on Endoplasmic Reticulum Stress in Model Plant Arabidopsis thaliana

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Background/Aim: Secretory and membrane proteins synthesized in the endoplasmic reticulum (ER) of eukaryotes must undergo proper folding and modifications. However, under stress conditions, they may either remain unfolded or misfold. This situation causes ER stress, prompting the cell to activate an adaptive signaling pathway called the Unfolded Protein Response (UPR) to cope with ER stress. ER stress can also be induced by the tunicamycin (Tm).
Glycyrrhizic acid belongs to the group of triterpenoids, which are mostly associated with regulating plant growth, development, and responses to the environment. Therefore, investigating the effect of glycyrrhizic acid on plants is crucial for understanding its potential effects in helping plants cope with stress conditions. This study aims to investigate the ER stress ameliorating effects of Glycyrrhizic acid in *Arabidopsis thaliana*.

Materials and Methods:*A. thaliana* ecotype Col-0 seeds were grown for 3 weeks in hydroponic culture with half-strength Hoagland solution. Plants were subjected to Tm (0.1 µg/ml) and Glycyrrhizic acid (50 µg/ml). The plants were harvested after 48 hours. Relative water content (RWC), leaf osmotic potential, leaf water loss, root phenotype analysis, H_2O_2 and Lipid peroxidation analysis were measured. The expression levels of the ER stress related genes were determined using qRT-PCR.

Results:48 hours after the application of tunicamycin, lipid peroxidation analysis showed 116.36% increase in plants treated with Tm compared to the control group. The group receiving both Tm and glycyrrhizic acid showed 11.76% improvement compared to the group receiving only Tm. In the H_2O_2 analysis, plants treated with Tm showed 99.99% increase compared to the control group. The group receiving both Tm and glycyrrhizic acid showed a 58.08% improvement compared to the group receiving only Tm.

Conclusion: Lipid peroxidation and H_2O_2 analyses indicate that glycyrrhizic acid treatment is beneficial for ER stress and it regulated the UPR gene network.

Keywords: Arabidopsis thaliana, Endoplasmic Reticulum Stress, Tunicamycin, Glycyrrhizic Acid, Unfolded Protein Response UPR

P206
Discovering Key DNA Damage Response (DDR) Factors for Radiotherapy Response in Glioblastoma Using the Novel CRISPR/Cas9 Library, DDRKOL

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Background/Aim: Cells encounter many factors that cause DNA damage and possess intricate DNA damage response (DDR) mechanisms to repair DNA injuries and maintain genomic stability. Defects in these pathways and accumulation of DNA damage result in genomic instability, a key hallmark of cancer, tumor formation and aging. Glioblastoma (GBM) is an aggressive primary brain tumor and typically treated with radiotherapy (IR) and chemotherapy (Temozolomide (TMZ)) post-surgery. IR Works by inducing DNA double-strand breaks (DSBs), which are highly lethal to cells and primary cause of cell death. This study aims to discover novel components of the DDR pathway that influence the response to IR in GBM using a novel CRISPR/Cas9 based screening technologies.

Materials and Methods: We developed the DNA Damage Response Knock Out Library (DDRKOL), a focused sgRNA library targeting 888 genes (10 sgRNA/gene) across all DDR pathways, curated from Gene Ontology Consortium, comprising 9000 sgRNAs. It also includes positive controls, non-targeting controls, and internal control genes.

Results: DDRKOL's composition and coverage were validated and used to investigate TMZ response in U87MG cells. Subsequent screens conducted in A172, U373 and IR-resistant U373 cell lines. In A172-DDRKOL screen, 2 Gy IR was applied, revealing PNKP, PPM1D, PPP1R10, PDS5B, COPS5, WDR33, WDR70, UBE2N and RNF113A as the most depleted genes when comparing initial timepoints to post-IR treatment. Further validation of these genes and identification of those most impactful on IR response are planned. Future screens will include IDH mutant and wild type A172 cells.

Conclusion: Identifying new genes that influence therapy response holds promise for overcoming therapy resistance and developing innovative cancer treatments with potential clinical application.

This study is supported by the TUBITAK 1001 program (#221S439).

Keywords: Glioblastoma, DNA Damage Response DDR, CRISPRCas9, Radiotherapy

P207
OMVs Derived from H. somni Induces Serum-Mediated Killing Against the Pathogen

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Aim: *Histophilus somni* is a Gram-negative pathogen causing thromboembolic meningoencephalitis, pneumonia, polyarthritis, acute myocarditis and urogenital system infections in cattle. Outer membrane vesicles (OMVs) are the spherical, non-replicative and highly immunogenic nanoparticles produced by the Gram-negative bacteria. These particles increase the microbial pathogenesis via delivering the virulence factors to the host efficiently, provide microbial resistance under the stress conditions, and activate the antigen-presenting host cells. They can induce humoral and cellular immune responses without a need to the pathogenic cell due to the high similarity of OMV surfaces and contents to the antigenic surface of the pathogen. Therefore, vesicles isolated from *H. somni* have been investigated for their immunomodulator properties and included in the content of vaccine development.

Materials and Methods: OMVs derived from *H. Somni* plantonic culture collected at late logarithmic phase of growth and inactivated whole cell *H. somni* injected into mice in two doses with 14 days interval with or without oil based adjuvant. Serum was collected with intracardiac puncture and used in subsequent Enzyme-Linked ImmunoSorbent Assay (ELISA) and Western-Blotting experiments as well as Complement-Mediated Bacterial Killing Assay.

Results: OMVs from *H. somni* able to induce detectable levels of immunoglobulin response and induces serum-mediated killing as comparable as inactive whole cell vaccine injection even when injected to mice at suboptimal protein concentrations.

Conclusion: OMVs derived from gram-negative pathogens are good candidates for next-generation vaccine platforms and presents ample opportunities in vaccination, and drug-delivery, in the context of medical and veterinary sciences.

Keywords: H somni, OMVs, Vaccine, Serum-mediated killing

P208
Dorsal Appendage Formation and Dorsal Closure in Drosophila melanogaster: A Versatile Model for Investigating Human Neural Tube Defects.

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Background/Aim: Neural tube defects (NTDs) are a range of malformations resulting from improper neural plate closure, influenced by genetic and environmental factors. Understanding NTDs is complex, necessitating model organisms with conserved molecular pathways. *Drosophila melanogaster*, though underutilized, is a potential model for NTD research. This study investigates dorsal closure (DC) and dorsal appendage (DA) formation in *Drosophila*, processes analogous to neural tube formation. We focus on the *Vang* gene, linked to planar cell polarity (PCP) and nervous system development, using flies with a *Vang* loss-of-function mutation.

Materials and Methods
Vangstbm-6 mutant lines were placed on sugar-containing agarose plates. After 14 hours, DA characteristics—thickness, length, and angle relative to eggs—were assessed using a Leica S8AP0 microscope.
Results: *Vangstbm-6* mutants exhibited defective DAs, including thin, malformed structures, contrasting with the well-formed DAs of Canton-S controls. Some mutants had completely absent DAs, while others showed short, thick, and malformed appendages.

Conclusion: This study reveals a crucial role of the PCP signaling pathway in DA formation and underscores the potential of *Drosophila* as a model for studying NTDs. Future research will involve detailed quantification of DA characteristics and live imaging to explore dorsal closure and epithelial patterning defects. This work advances our understanding of genetic factors contributing to NTDs and could inform therapeutic strategies.

Keywords: Neural tube defects, Planar cell polarity, Vang, Dorsal appendage, Drosophila melanogaster

P209
Do We Know The Effect Of Substance Use On Cell Metabolism?

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Background/Aim
Drug and stimulant use creates major health and social problems. The most commonly used substances in our country are amphetamines. This is followed by cannabis and opiate derivatives. Unfortunately, during clinical follow-up of drug users, it is not clear how many users are exposed to cancer in the following years.And it has not been fully established how much amphetamine, marijuana, opiate or cocaine addiction increases susceptibility to cancer. There are not enough scientific articles on whether these substances have a proliferative, cytotoxic or apoptotic effect on the cancer cell. Our aim with this declaration is to identify the most used substances in our drug and stimulant measurement laboratory.Materials and Methods28456 samples for which urine sample analysis was requested from our laboratory were retrospectively screened. Substance screening requests were made for forensic cases, medical board patients or emergency patients. All screening analyzes were performed with the LC-MSMS (Sciex 4500) device. Results48% of these samples were positive for at least one drug or stimulant. 50% of the samples were positive for more than one substance. Among the positive results, the most common substance is amphetamine and ecstasy (%65,3). This is followed by cannabis, morphine and pregabalin, respectively. ConclusionIn general, in the field of health, the clinical effects of these banned substances are widely debated. However, the molecular mechanisms of these clinical effects are not adequately discussed. Moreover, since most patients do not come for treatment or their health problems,follow-up cannot be done. Normally, the primary goals of clinicians may be to detect and treat substance use. However, the point we want to draw attention to here is this; What kind of effects do these substances have on cell biology and metabolism? Does it have a proliferative effect on cancer cells? Or is it apoptotic?

Keywords: drug abuse, amphetamins, biological effects, opioids, cannabinoids

P210

Comparison of Endoplasmic Reticulum Stress Tolerance in the Solanaceae Family and Their Wild Relatives

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Background: Protein folding occurs in the endoplasmic reticulum (ER) in eukaryotes. Stress conditions diminish this process and secretory proteins cannot fold properly and these unfolded polypeptides accumulate in ER lumen. This condition is known as ER stress and can be lethal. A specific signal and regulatory mechanism, called as the unfolded protein response (UPR) is activated to regulation of ER. ER stress can also be specifically induced by agents like antibiotic tunicamycin (Tm). This study aims to compare the physiological and transcriptomic responses to ER stress in tomato and pepper varieties belonging to the Solanaceae family with an emphasis on to set ER stress response as criteria for selection in field crop trials.

Materials and Methods: The seeds were germinated in petri dishes including control Tm groups. Germination rates were evaluated for all the genotypes. The relative water content (RWC), leaf osmotic potential, and leaf water loss. Root phenotyping analyses were also performed. Using quantitative real-time PCR (qRT-PCR), the expression of the genes *ERO1, BZIP17, BZIP28, BZIP60, IRE1A, IRE1B, BiP1, BiP3, CNX, HRD1, SEL1, DER1,*and *UBC32* were determined.

Results: The results showed that, among the tomato varieties, MSC50, SC2121 was found to be resistant to Tm treatment as evident by root phenotyping analyses. This variety also showed enhanced UPR gene transcript under stress conditions which can be evaluated as a good candidate for field trials. pepper was enclosed as the more robust variety which also showed increased levels of ER associated degradation genes that can be targeted for further analysis.

Conclusion: Abiotic and biotic stress tolerance can be evaluated by different criteria's in crop growth. We suggested that ER stress tolerance can be counted as such selection criteria. For further analysis, field trials will be conducted under ER stress with this promising tomato and pepper varieties for further evaluations.

Keywords: Endoplasmic Reticulum Stress, Solanaceae Family, Unfolded Protein Response UPR, Tunicamycin TM

P211
Dynamic Recognition of The Nucleosome Core Particle by Select Chromatin Factors

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Background/Aim: The eukaryotic genome is organized by chromatin, a complex of proteins and nucleic acids. The nucleosome core particle, the smallest unit of chromatin, consists of two copies of each core histone protein (H2A, H2B, H3, and H4) and around 145 base pairs of DNA. Nucleosomes interact with various chromatin factors and enzymes, highlighting the importance of multivalency in binding and specificity. This study uses molecular modeling and high-performance computing to examine the shared characteristics of nucleosome recognition by five different chromatin factors, which have diverse functions but similar chromatin binding mechanisms.

Materials and Methods: We use atomic coordinates of five nucleosome-bound chromatin factors SAGA-DUB, Sir3, PRC1, RCC1, and 53BP1 from the Protein Data Bank (PDB) to construct computational models. These models are validated by aligning the DNA sequences using Clustal Omega and Web 3DNA 2.0. Unresolved disordered loops are identified and corrected using PDB annotations, SuperLooper2, and PyMOL. Each model is solvated in a cubic water box with NaCl and Mg ions, described using the CHARMM36m force field and OPC water model. For each model, 100 ns production trajectories are collected using Gromacs 2018 and analyzed with in-house Python scripts, PyMOL, and VMD.

Results: All five chromatin factors bind to the two faces of the nucleosome core particle in different ways. These differences in binding mode are associated with the varying degrees of local stability of the chromatin factors, which are determined by the flexibility of their loop domains. We also characterize the intricate interactions between the acidic patch of the nucleosome core particle and the arginine anchor motifs of the chromatin factors.

Conclusion: We use molecular modeling and high-performance computer simulations to investigate the major advances in biophysical studies of the interactions between chromatin-binding proteins and the nucleosome core particle. We reveal how these molecular machines access DNA to carry out their various biological functions.

Keywords: chromatin, nucleosome, molecular dynamics, computational biophysics

P212
Regulation of Protein Post-Translational Modifications by Interaction of Cellular Proteins in Yeast

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Background/Aim: Different post-translational modifications could be observed by protein migration pattern on western blotting and are essential for proper cell cycle progression in yeast. This study aimed to explore how interactions between specific cellular proteins influence the appearance of protein bands, particularly in mutant yeast strains.

Materials and Methods: Yeast strains with specific gene deletions were transformed with plasmids containing either wild-type or mutant versions of the genes of interest. Protein bands were analyzed using western blotting. Cell cycle stages were also manipulated using specific inhibitors to study protein bands at different phases.

Results:A significant alteration in the protein migration pattern was observed in mutant yeast strains. Partial restoration of the normal protein band pattern occurred when the mutant strains were transformed with wild-type genes, while complete restoration was observed with certain mutant gene versions. These findings suggest that specific protein interactions play a critical role in regulating the appearance of protein bands. No alteration in protein bands was observed during specific cell cycle arrests, indicating a cell cycle-dependent regulation.

Conclusion: Interactions between specific cellular proteins are crucial for regulating the appearance of protein migration patterns in yeast. Disruption of these interactions can significantly impact protein bands, highlighting the importance of protein-protein interactions in cell cycle regulation.

Keywords: protein migration pattern, cell cycle, protein interactions, gene mutations

P213
Searching the Effect of Adiponectin on CD36 Expression in H9c2 Cell Line

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Background/Aim: Diabetic cardiomyopathy is a structural and functional myocardial disorder associated with diabetes that is not associated with the traditional factors. In the healthy heart, glucose and long-chain fatty acids are used equally as energy sources. There is a dynamic balance in their uptake and transport. Adiponectin (APN) is an adipokine synthesized and secreted by adipose tissue. APN concentration is reduced in insulin resistance and type 2 diabetes. APN prevents insulin resistance by increasing fatty acid excretion and glucose by skeletal muscle. CD36 plays a role in lipid metabolism and signal transduction. It facilitates the cellular uptake of LCFA in the plasma membrane, especially in the heart and skeletal muscles. Blocking CD36 in cardiomyocytes results in the prevention of excessive lipid uptake.The aim of this study is to search the effect of adiponectin on changes in gene expression responsible for fatty acid uptake and transport in cardiac cell in vitro.

Materials and Methods: Immunofluorescence (IF) staining was performed with demonstrated primary and secondary antibodies for cardiac-specific myosin, muscle-specific desmin intermediate filaments, and specific granules containing perinuclear Atrial Natriuretic Factor (ANF) to demonstrate cardiac behaviour of H9c2 cell line. H9c2 cardiac in vitro cell model that mimicking diabetic cardiomyopathy was created and the effect of adiponectin on the expression changes of CD36 gene under basal, hypoglycemic and hyperglycemic conditions was determined by Western blot.

Results: In the IF study showed H9c2 cell line's cardiac behavior. Time-dependent treatment of APN in different concentrations to H9c2 cells resulted in changes in CD36 expression. From this, it can be suggested that APN may be effective in fatty acid translocation.

Conclusion: This study aims to define new mechanisms that play a role in the pathogenesis of Diabetic cardiomyopathy.

Keywords: Adiponectin, Diabetic cardiomyopathy, H9c2 cell line, CD36

P214
Transcriptomic Characterization of TCR-NK Cells Reveals Challenges Associated with CD3ζ Overexpression

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Cytotoxic T cell activity can be redirected towards tumor antigens by T cell receptor (TCR) gene therapy. One challenge to be addressed in this approach is the "mispairing" issue which results in mixed TCR dimers with unknown specificity. In order to solve this issue, our lab has already demonstrated the proof-of-concept that relies on NK cells that have been genetically altered to express functional TCRs (TCR-NK cells). TCR-NK cells that have expressions of CD3δ, CD3γ, CD3ε, CD3ζ and TCRα/ß can recognize an antigenic peptide presented by MHC-I and exhibit cytotoxic activity specific to that antigen. However, we have observed that the overexpression of CD3ζ in TCR-NK cells appears to boost background activity against non-specific targets. We aimed to comprehend the transcriptomic alterations linked to genetic modification by thoroughly examining the gene expression profiles of TCR-NK cells, to identify possible causes of non-specific activity resulting from the inclusion of CD3ζ.

Following lentiviral transduction of the CD3 complex (CD3δ, CD3γ, and CD3ε), either with or without the CD3ζ and TCRα/ß genes, bulk RNA sequencing was carried out to compare genetically modified and wild type NK92 cells. Our data demonstrates that all genetically modified cells had markedly altered genes involved in antiviral defense mechanisms. Apart from these genes, the addition of TCRα/ß and the CD3 complex (without CD3ζ) didn't result in many notable changes. However, the profiles of cells that overexpress CD3ζ differ significantly. With merely the inclusion of the CD3ζ gene, over 600 genes were dramatically up- or down-regulated, which may account for the previously stated background activity against non-specific targets.

We conclude that the antigen-specificity of TCR-NK cells may be negatively impacted by the vector design's incorporation of CD3ζ. Only the CD3δγε components of the CD3 complex should be expressed in order to support the continued growth of TCR-NK cells.

Keywords: TCR-NK, Immunotherapy, NK Cells, T Cells

P215
Cellular Localization and Subcellular Translocation of Fluorescent Protein Labelled CCDC124 Protein Under Stress Conditions

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The CCDC124 gene is conserved throughout eukaryotic organisms. Ccdc124 protein locates within centrosomes and midbodies. Similar to the majority of centrosome proteins, it contains a CCD region. In interphase, it is present in the centrosome, and during telophase it translocates to the midbody. CCDC124 is composed of a total of 223 amino acids. Through the use of mass spectrometry analysis, the mass of CCDC124 was determined to be approximately 32 kDa.

Numerous protein interactions and biochemical reactions occur simultaneously in confined spaces inside eukaryotic cells. Organelles defined by phospholipid membranes provide relatively limited spaces that allow efficient and specific progression of various signaling pathways and biological interactions. Biomacromolecules such as proteins and nucleic acids can form liquid-like membrane-less condensates through liquid-liquid phase separation (LLPS), which provides an alternative way of concentrating and separating cellular components in a spatially defined manner for various functional processes. DYRK3 is a protein that forms clusters under stress conditions through LLPS. It was discovered to be an interaction partner of CCDC124 following the mass-spectrophotometry results. Based on the data, the objective was to utilize microscopic methods to identify the localization and translocation of CCDC124 in cells. Additionally, the aim was to explore whether there is a functional interaction between DYRK3 kinase and CCDC124 through studies involving a DYRK3 inhibitor.

The U2OS (Human Bone Osteosarcoma Epithelial Cells) cells were transfected using Lipofectamine™ 3000 Transfection Reagent (Invitrogen™). Organelle-targeted plasmids were used to examine their interactions with organelles. The imaging procedure for the control groups commenced 24 hours after transfection. DRYK3 plasmids were used for stress granule experiments. For the oxidative stress construct, the cells were exposed to 500 µM arsenite for 45 minutes. To induce osmotic stress, cells were treated with 0.4 M sorbitol for 45 minutes. The prepared CCDC124 constructs were observed under Zeiss-Lsm 780-980 and Lattice Light Sheet 7 microscopes.

Keywords: CCDC124, LLPS, Mıcroscopy, Mitochondria, Stress Granule

P216
Simultaneously Targeting Ezrin and ERG in Lung Cancer Cells: A promising idea

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Background/Purpose: ETS related Gene (ERG) is a member of ETS family proteins and it has been shown that ERG is essential for endothelial homeostasis, differentiation, and angiogenesis. Several ERG targeted genes have been implicated in angiogenesis, and it has been shown that Erg-dependent expression of VE-cadherin is essential for endothelial junctional stability and endothelial survival, both critical processes in angiogenesis. Ezrin is a protein that has recently attracted attention with its oncogenic properties and is known to play a role in cell motility and signal transduction. A study has shown that ezrin affects the function of the transcription factor ERG. The aim of this study is to investigate the effect of the combined use of ezrin and ERG inhibitors on lung cancer cells.

Materials and Methods: ERG inhibitor YK-4-279 and ezrin inhibitor NSC668394 used separately and in combined forms to evaluate cytotoxicity, motility and colony formation ability of A549 cells. Western blot and rtPCR were performed to determine the expression levels of genes associated with apoptosis, the cell cycle, and EMT.

Results: ERG and ezrin protein levels are screened in various lung cancer cell lines. Both ERG and Ezrin are expressed at high levels in A549 cells. YK-4-279 and NSC668394 had IC50 values of 3uM and 5uM respectively. Inhibitors showed a synergistic effect in cell killing and motility and suppressed the expression of genes associated with EMT and apoptosis strongly.

Conclusion: Simultaneously targeting ERG and Ezrin appears to be a potent approach for suppressing the oncogenic characteristics of cancer cells. Upcoming in-vivo research will be conducted in the near future.

Keywords: Ezrin, ERG, A549, Synergy

P217
Genetic Screening to Identify Novel Gene Interactions in Between the Mitotic Exit Network and PI(3,5)P2 Pathways in Yeast Mitotic Network Pathway

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Background/Aim: In budding yeast (Saccharomyces cerevisiae), the mitotic exit network (MEN) regulates the transition from mitosis to the G1 phase, ensuring proper cell division by coordinating the inactivation of mitotic cyclin-dependent kinases and the completion of cytokinesis. Phosphatidylinositol 3,5-bisphosphate (PI(3,5)P2), an essential membrane lipid produced by the lipid kinase Fab1, regulates MEN, and its activity relies on Vac7 and the regulator Vac14. Deletion of VAC7 or VAC14 reduces PI(3,5)P2 levels, and VAC7 deletion in MEN-defective cells causes lethality. This study aims to identify genes whose overexpression can rescue this synthetic lethality, providing insights into this regulatory pathway.

Methods: A strain with a conditional loss-of-function mutant of MOB1, a MEN protein, combined with vac7Δ, will be transformed with a yeast overexpression genome library. Colonies will be selected, and their plasmids will be isolated and amplified through yeast plasmid isolation and E. coli transformation. Sequencing will identify the genes.

Results: Screening identified several colonies with robust growth at restrictive temperatures, suggesting successful rescue of synthetic lethality. These colonies have been isolated, and the corresponding plasmids will be sequenced to identify the responsible genes.

Conclusion: This genetic screening will identify genes that rescue synthetic lethality between MEN mutants and PI(3,5)P2 levels, enhancing our understanding of genetic interactions within the MEN pathway and providing insights into cell division.

Keywords: Overexpression rescue, genetic screening, Mitotic Exit Network, Synthetic Lethality

P219
Investigation of Bag-1S:p97/VCP Interaction in Breast Cancer Cell Line

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Background/Aim: Proteostasis includes all protein maintenance processes and involves in protein quality control (PQC) mechanisms such as molecular chaperones, endoplasmic reticulum-associated degradation (ERAD), and ubiquitin/proteasome system (UPS). Bag-1 protein, which is upregulated in breast cancer cells, consists of two functional domains: BAG and ubiquitin-like (UbL). Additionally, Bag-1 has multiple interaction partners, determining its effect on proteostasis. It is known to be involved in protein folding and degradation processes through interactions with Hsp70 and UPS. AAA+ ATPase p97/VCP protein enables the separation of substrate proteins from the cellular structure or multi-protein complexes by ATP hydrolysis, allowing proteins to be targeted to the proteasome for destruction. Studies conducted in our laboratory had shown that Bag-1S protein directly interacts with p97/VCP protein, and thus has a role in ubiquitin-dependent degradation. In this study, it was aimed to understand the importance of the Bag-1S:p97/VCP complex in protein homeostasis.

Materials and Methods: His_6TEVBag-1S and His_6TEVp97 were transfected separately and together to MCF-7 breast cancer cells. The expression levels of proteins related with Bag-1S:p97/VCP complex were investigated. After overexpression of the proteins, protein synthesis inhibitor Emetine was applied to the cells at different times, and expression levels of ERAD substrate was investigated by immunoblotting.

Results: Our results showed that, expression levels of apoptosis marker PARP was reduced when the Bag-1S:p97/VCP complex was overexpressed. Moreover, Bag-1S overexpression reduced the ERAD activity.

Conclusion: It is important to understand the effect of overexpression of wild-type Bag-1S and p97 proteins on protein homeostasis in MCF-7 cells. In the light of our ongoing studies, the relationship between Bag-1S and p97/VCP proteins will become clear and their importance in protein degradation mechanisms will be emphasized.

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Keywords: Bag-1S, p97/VCP, ERAD, protein homeostasis

P220
Combined Effects of Antifibrotic agent Nintedanib and Ezrin Inhibitor on Lung Cancer Cells co-cultured with cancer associated fibroblasts

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Background and aim: Nintedanib is a tyrosine kinase inhibitor that has been using in pulmonary fibrosis treatment and also has ability inhibit cell proliferation in cancer cells. Ezrin is a member of the ERM protein family (Ezrin, Radixin, Moesin) and contributes to cell shape maintenance, cell motility, cell signaling and cell division by connecting the cell membrane to actin filaments. Ezrin also leads collective invasion and EMT process in cancer cellsç The use of Ezrin inhibitors against cancer cells is important in suppressing these biological processes. This work aimed to determine the effects of Ezrin inhibitor and Nintedanib on migration of A549 cells co-cultured with primary fibroblasts.

Methods: A549 cells and primary cancer associated fibroblasts obtained from a lung cancer patient were maintained in DMEM supplemented with 10% FBS. IC50 values of ezrin inhibitör and nintedanib were determined by MTT. The migratory effects of ezrin inhibitor and nintedanib on co-cultured A549 and primary fibroblast cells were determined by wound- healing assay. Fluorescent staining was performed to visualize collective invasion areas.

Results: The IC50 values of Nintedanib and Ezrin inhibitor in A549 cells were determined as 9.02 µM and 66.91 µM, respectively in co-cultured cells. Ezrin and Nintedanib inhibited cell migration in co-cultures established with A549 and primary fibroblast. When applied together, the compounds increased cell death as well as significantly suppressed motility of cancer cells.

Conclusion: Antifibrotic agent nintedanib and inhibiton of ezrin might be a useful tool to suppress cancer cell invasion and metastasis. In-vivo studies are needed to further evaluate the effects of this combinational treatment model.

Keywords: Nintedanib, Ezrin, lung cancer, cell motility

P221
Long Non-Coding RNAs in CD4 T Cell Activation: Enhancing Immunotherapy Through Bioinformatics Analysis

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Background/Aim: CD4 T cell activation in the tumor microenvironment is crucial for enhancing the efficacy of immunotherapies such as checkpoint inhibitors. In vitro activation of T cells is vital for selecting, expanding, and genetically modifying T cells for therapeutic purposes. Recently, long non-coding RNAs (lncRNAs) over 200 base pairs have gained attention for their potential effects on T cells. The impact of different T cell stimulatory conditions on lncRNAs is an unexplored area.

Materials and Methods: In this project, CD4 T cell microarray data (GSE39594) from human blood samples were analyzed under different stimulatory conditions (non-stimulated, anti-CD3, and anti-CD3 plus anti-CD28) at different times (0, 4, and 24 hours). Differential gene expression analysis using the Limma package in R identified lncRNAs that might be responsible for these differences. Target lncRNAs, potential biomarkers, were investigated using single-cell data with the Seurat package in R to understand their effects on CD4 T cells in the tumor microenvironment. Examining various lncRNAs involved in T cell activation using advanced single-cell RNA sequencing methods might provide insights for clinical studies. This approach helps identify different CD4 T cell subsets in terms of lncRNAs, benefiting immunotherapy responses.

Results: Bioinformatics analyses have shown the potential lncRNAs that might be responsible for the different T cell activation levels between the combined use of CD3/CD28 beads and using only CD3 or control samples and different hours. Various lncRNAs might be responsible for these activation changes, highlighting potential biomarkers. Some candidate lncRNAs were observed in different subsets from single-cell data.

Conclusion: This study identified lncRNAs that might be associated with different T cell activation methods and incubation times, potentially improving immunotherapy outcomes by revealing insights into CD4 T cell subsets and activation mechanisms. The findings suggest that specific lncRNAs may serve as biomarkers, enhancing the understanding and efficacy of immunotherapies.

Keywords: bioinformatics, cancer, lncRNAs, immunotherapies

P222
Uncovering LncRNA Heterogeneity In Activated T-Cells

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Background/Aim: The research on activated T-cells has played a crucial role in uncovering the complexity of various biological events, including phenotypic alterations, cell signaling, and T-cell variance. The involvement of long non-coding RNAs (lncRNAs) in regulating T-cell responses remains understudied. RNA transcripts of 200+ nucleotides that do not encode proteins are called lncRNAs. The regulation of expression of genes, differentiation in cells, development, and physiological processes are affected by lncRNAs. Each T-cell type has different features; thus, it is thought that both protein-coding genes and non-coding RNA variation add to the creation of this spectrum of T-cells. T-cells play important roles in directly removing malignant cells and contributing to a variety of disease pathologies. We aimed to elucidate the heterogeneity in lncRNA expression during T-cell activation in a time-dependent manner.

Material/methods: Analysis of GEO197067 data involved activating Pan T-cells from healthy donors with anti-CD3/CD28 beads at multiple time points. Using the limma package in R, we analyzed differential gene expression, created heatmaps, and gene set plots, and compared them to other pathways. Created T-cell activation gene sets were validated on single-cell RNA sequencing (scRNAseq) CD4/CD8 T-cell data.

Results: The data has over 2.5k lncRNAs, with around 600 showing differential expression between activated and non-activated timepoints. Some lncRNAs are differentially expressed at specific time points, while others are consistent across all time points. These were grouped into early and late activation and analyzed in cancer scRNAseq data to explore tumor-infiltrating T-cell patterns, especially in further subgroups such as CD4/CD8 T-cells.

Conclusion: Identification of nearly 600 lncRNAs that were differently expressed according to the activation status and timepoint was shown and these lncRNAs displayed different patterns when categorized into early/late activation groups. Overall, this work demonstrated that lncRNA expression patterns during T-cell activation are highly heterogeneous.

Keywords: Immunology, T Cell Activation, LncRNA, Differential Gene Expression

P223
Investigating the Intersection of LINE-1 Retrotransposition and DNA Replication

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Background/Aim: Long interspersed element 1 (LINE-1, L1) is the only active retrotransposon in modern humans and its aberrant overexpression is found in nearly half of human cancers. L1 generates *de novo* insertions of itself via a 'copy-and-paste' mechanism that is dependent on two protein-coding open reading frames (ORFs): ORF1p, an RNA-binding protein and ORF2p, an endonuclease (EN) and reverse transcriptase (RT). Accumulating evidence implies that the insertion of L1 into the genome occurs during S phase and that DNA replication and replication-coupled repair factors limit retrotransposition. Additionally, L1 expression generates molecular vulnerabilities to the loss of DNA replication and repair factors. These synthetic lethal interactions suggest a model wherein L1 retrotransposition intermediates hinder DNA replication progression, a condition referred to as DNA replication stress (RS), that can lead to fork collapse, DNA damage, and consequent genomic instability.

Materials and Methods: We performed state-of-the-art cell biology and biochemical methods combined with advanced molecular imaging techniques

Results: Using DNA fiber assays, we found L1-expressing cells display a marked reduction in replication fork velocity accompanied with an asymmetric pattern of sister replication tracts, an indication of direct fork stalling. Intriguingly, we observed higher frequency of newly fired DNA replication origins in L1-expressing cells. We found that inhibition of new origin firing completely rescued L1-induced RS, suggesting its dependence on aberrant origin firing. Mitotic entry with persistent replication intermediates through S/G2 checkpoint triggers a mitotic DNA synthesis (MiDAS) and compromises chromosome segregation, causing chromosomal breakage and genome instability. Indeed, we found higher frequencies of MiDAS and cytological markers of chromosome missegregation upon L1 expression.

Conclusion: These findings suggest that L1 expression impairs replication fork progression, causing replication stress, leading to mitotic defects and DNA damage in the daughter cells. Our findings are expected to reveal the molecular mechanisms underlying L1-associated DNA damage and genome instability, a hallmark of cancers.

Keywords: LINE-1, replication stress, genomic instability, DNA repair, retrotransposition

P224
Biological Control Potential of Bacillus pumilus: An prelimary study in Pectobacterium carotovorum (Erwinia carotovora) soybean disease model

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Aim: *Bacillus pumilus* is an important candidate in the fight against plant pathogens by inhibiting the quorum sensing (QS) mechanisms of some microorganisms. *Pectobacterium carotovorum* is a gram-negative bacterium that causes soft rot disease in plants by producing acyl-homoserine lactone (AHL) molecules through QS. In this study, we investigated the potential of *B. pumilus* to inhibit the pathogenicity of *P. carotovorum* in soybean disease model.

Material-Method: Plates impregnated with *B. pumilus* on filter papers were added to Kings B agar inoculated with *P. carotovorum,* and inhibition zones was observed. To measure the pathogenicity, biofilm formation capacity of the bacteria was tested by crystal violet staining in the presence of *B. pumilus* cultures. *P. carotovorum* culture was infiltrated to the leaf lamina of soybean. The infection and disease formation was observed and scaled. The inhibitory effects of *B. pumilus*spraying in the number of lesions formed by infiltration were examined. Photosynthetic pigments, anthocyanin and flovanoid content and MDA levels were measured and compared between the study groups.

Results: According to the results obtained, in vitro, *B. pumilus* and *P. carotovorum* showed inhibition on each other. To evaluate these effects in vivo, *P. carotovorum* was applied to soybean leaves as an infectious agent and disease symptoms were monitored. Following the first symptoms, *B. pumilus* was applied. Differences were found in photosynthetic pigments, anthocyanin and flovanoid content, MDA amounts between the infected group, infected+biological control agent group and non-infected groups.

Conclusion:
This study demonstrated the potential biological control potential of *Bacillus pumilus* in soft rot disease of soybean caused by *Pectobacterium carotovorum*. Although the molecular mechanisms underlying biological control and QS regulation will be further investigated, these results demonstrated the optimization and usefulness of the disease model.

Keywords: Pectobacterium caratovora, Bacillus pumilus, Biological control

P225
Comparative Analysis of the Bs2 Gene in Tomato and Soybean: Insights into Resistance Against Xanthomonas gardneri

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Aim: *Xanthomonas gardneri* is a gram-negative aerobic bacterium that causes bacterial spot disease in most crops of economic value such as tomato, pepper, cotton, beans and soybeans. The *Bs2* gene is known to confer resistance to *Xanthomonas* species in pepper and tomato. In this study, the properties of the *Bs2* gene in tomato and the *Bs2* gene in soybean are compared by *in silico* methods and the resistance of soybean against *Xanthomonas* is evaluated.

Material and methods: Genomic, transcript and protein sequences of the *Bs2* gene of tomato and soybean were downloaded. Chromosome and exon-intron maps, chromosome locations, protein motifs, 3D models and phylogenetic analyses were analyzed comparatively between tomato and soybean. Chlorophyll measurement, anthocyanin and flavonoid content were also determined to evaluate the effect of infection with
Xanthomonas gardneri on photosynthetic activity and defense systems to correlate with bioinformatics data.

Resutls: The *SlyBs2* gene in the tomato genome has some functional genomic similarities with *GmBs2* in soybean. Both genes contain the same number of exons. According to GO results, the gene in soybean has molecular functions similar to those in tomato, such as involved in regulation of defense, involved in regulation of jasmonic acid mediated signaling pathway and involved in response to wounding. Although the protein motifs, 3D models and isoforms of both genes differ (*SlyBs2* has 2 isoforms, *GmBs2* has 4 isoforms), the results show that they are physiologically similar.

Conclusion: Having the same exon region indicates that the genes have a common evolutionary origin and are functionally similar. Both genes have some commonalities and differences. Ontological and phylogenetic similarities are *in silico* proof that *GmBs2*'gene will show resistance against *Xanthomonas*, even though they are different organisms. The data obtained show the diversity and adaptability of plants.

Keywords: Xanthomonas gardneri, Glycine max, Bacterial spot, biotic stress

P226
In-Silico Rational Mutation and Affinity Maturation of anti-CD19 Nanobodies

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Background/Aim: B-cell Acute Lymphoblastic Leukemia (B-ALL) involves the uncontrolled proliferation of B-lymphocytes. Antibodies or antibody fragments specific to CD19, a B-lymphocyte antigen, are used in B-ALL diagnosis and treatment. Nanobodies, the variable region (VHH) of heavy chain-only antibodies, retain all characteristics of whole antibodies in binding to antigens. This project aims to design nanobodies with high affinity for CD19 utilizing multiple in-silico maturation paradigms.

Materials and Methods: Two nanobodies (NBs) from H. Wang et al., 2021, with affinity to CD19, were selected. The 3D structures of CD19 and anti-CD19-NBs were predicted using ColabFold. Protein-protein interactions between CD19 and anti-CD19-NBs were predicted using an in-house pipeline with HDOCK. Mutations were introduced primarily in the CDR regions of the NBs using statistical, sequence-based, and structural considerations. The highest affinity mutants were selected based on interaction scoring methods like Bluues2 and binding energy calculations using MM/PBSA.

Results: 3D structural models of two anti-CD19-NBs were predicted using ColabFold, and HDOCK-predicted protein-protein interactions with CD19 were analyzed. Four matured anti-CD19 variants were chosen with improved binding free energy scores, ranging from -45 kJ/mol to -195 kJ/mol over the original NBs, based on MM/PBSA binding free energy calculations from 50-ns molecular dynamic simulations.

Conclusion: Affinity maturation increased the binding efficiency of the NBs and produced favorable physiochemical parameters for in-vitro investigation. The binding and specificity of newly designed NBs will be verified in vitro.

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Keywords: B-ALL, CD19, Nanobodies, In-silico affinity maturation

P227
Synthesis and In-silico analyses of new Malononononitrile Compounds to target Ezrin Y146 Site

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Background/Aim: Ezrin is a protein that regulates interaction between the cytoskeleton and the plasma membrane. It plays a critical role in cell shape, movement and signal transduction. Current research mostly focusses on ezrin inhibitors that target T567 site of the protein but the protein also has different phosphorylation sites. Amon them, Y146 (tyrosin146) region of ezrin is important in nuclear translocation of EGFR. In silico methods such as molecular docking are important for designing and evaluating the drugs before proceeding to expensive in-vitro and in-vivo studies. In this study, we evaluate the efficacy of novel inhibitors by focusing on the Tyr146 site of ezrin through molecular docking methods.

Materials and Methods : Carbostryl-selenophene hybrid compounds labelled as 20a, 20b, 20d, 20e, 20f, 20g, 20h synthetized and converted to malononononitrile compounds by Gewald reaction. The structures of ezrin protein that includes Y146 sites were obtained from the Protein Data Bank with the codes 4RM8 and 1NI2. 3D compounds were cleaned from water and hetam molecules using mgltools and docking analyses were performed using Autodock4 and mgl tools software to examine the interaction with Ezrin Y146 and the 7 ligands.

Results: The binding energies of 7 compounds with 4RM8 form of Ezrin were between -6.73 kcal/mol and -5.88 kcal/mol and with 1NI2 were between -7.31 kcal/mol to -5.37 kcal/mol. According to these results, 7 ligands generally have lower binding energy for 1NI2, and 20a had the most promising compound to target Y146 Ezrin.

Conclusion: This study is a critical step towards the discovery of new inhibitors directed against the Tyr146 site and evaluation of their efficacy.

Keywords: drug design, ezrin, molecular docking, Melanononitrile compounds

P228
Investigation of the Effect of HIF1α and HIF1ß on Collective Invasion in Lung Cancer Under Hypoxic Condition

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Background/Aim: Non-small cell lung cancer (NSCLC), accounts for approximately 85% of lung cancer, is still a big patients yet remains at a low level which is predominantly attributed to tumor metastasis. Tumor metastasis can be depend on many factors in cells. Hypoxia-inducible factors (HIFs) are key elements for controlling immune cell metabolism and functions. It is aimed to understand the difference between HIF1α and HIF1ß gene knockdown under normoxic and hypoxic conditions.

Materials and Methods: A549 cell line were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% Fetal Bovine Serum (FBS) at 37 °C and 5% CO2. Cells were seeded on plates for protein isolation and wound-healing assay. HIF1α and HIF1ß genes were knocked down with siRNA and the control group were examined under hypoxic and normoxic conditions. The effect of scratches on collective invasion after 24 hours was measured with the ImageJ program. The expression levels of GLUT1, HER2 and Ezrin were measured in the isolated proteins by Western Blot.

Results: According to the measurement results of the ImageJ program, the level of collective invasion under hypoxic conditions increased by 31.77% in HIF1α knockdown, while it decreased by 28.28% in HIF1ß knockdown. According to Western blot and ImageJ Quantification results, Ezrin expression increased by 22.71% and 28.33% Her2 expression increased by 13,51% and 16,74% in HIF1α knockdown and HIF1ß knockdown, respectively.

Conclusion: Ezrin proteins locate in cellular structures where the plasma membrane proteins interact with the actin cytoskeleton and maintain the structural stability of cells. In HIF1α knockdown, our data supports the correlation between collective invasion and ezrin protein expression levels. Otherwise, for HIF1ß knockdown was not related with protein expression of ezrin.

Keywords: collective invasion, lung cancer, hypoxia, gene knockdown, hypoxia-inducible factor

P229
Synergistic effect of Dacomitinib and Olaparib combination on Gemcitabine-Resistant Triple Negative Breast Cancer MDA-MB-231 cell line

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Introduction and Aim: Breast cancer is the leading cause of cancer-related deaths in women, and one of its most challenging subtypes is Triple Negative Breast Cancer (TNBC). TNBC has limited targeted therapies and poor prognosis due to the absence of receptor expression and often develops resistance to chemotherapy. Epidermal Growth Factor Receptor (EGFR) and Poly (ADP-ribose) polymerase (PARP) pathways are known to control cell proliferation, growth, survival and metastasis in breast cancer. Dacomitinib is an irreversible pan-HER inhibitor and combination approaches other inhibitors has the potential to overcome innate resistance. 10-15% of TNBC tumors carry hereditary mutations in BReast CAncer early onset 1 and 2 genes (BRCA1/2 genes). This study aimsto find potential therapeutic through combined treatment of EGFR and PARP inhibitors to overcome drug resistance in TNBC.

Materials and Methods: Gemcitabine-resistant MDA-MB-231 (MDA-MB-231^{GEM}) cell line was developed by treating with increasing doses of gemcitabine over a year. Resazurin assay was used to evaluate the cytotoxic effects, while colony formation and scratch assays were conducted to assess the clonogenicity and migratory capacity, respectively. Gene expressions were evaluated by q-PCR and cell cycle assay was used to detect the cell distribution.

Results: The effective combination treatment dosage on MDA-MB-231^{GEM} cells was found to be 1.5 µM Dacomitinib and 1.5 µM Olaparib for 48 hours. The combination treatment of Dacomitinib and Olaparib on MDA-MB-231^{GEM} cells reduced migration and colony formation compared to the control group. Combination therapy shows a decrease in EGFR, PARP and mTOR gene expression levels and G1-phase cell cycle arrest in MDA-MB-231^{GEM} cells.

Conclusion: Our study demonstrated that the combination of Dacomitinib and Olaparib exhibits anti-proliferative effect on MDA-MB-231^{GEM} cells. These findings suggest that this combination therapy could be a promising anti-cancer agent to treat gemcitabine-resistant breast cancer.

Keywords: Triple negative breast cancer, Olaparib, Dacomitinib, Gemcitabine-resistant

P231
Generation and genetic characterization of genetically engineered mouse models for a neurodevelopmental rare genetic disorder

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Autophagy is a fundamental eukaryotic cellular process that degrades and recycles large cellular components, maintaining cellular homeostasis under normal or stress conditions. It is regulated by a set of proteins including ATG (autophagy-related gene) proteins, which orchestrate the formation and maturation of autophagosomes. Dysfunction of autophagy may result in human pathologies including cancer and neurodegenerative diseases. Additionally, autophagy plays an important role in trophoblast migration/invasion in human placental development and has been shown to be mis-regulated during preeclampsia. Mendelian diseases associated with ATG genes are rare. Mutations in core autophagy genes ATG5 and ATG7 have been previously reported to cause rare genetic disorders with autosomal recessive inheritance pattern. We previously identified a mutation in the placenta specific expressed ATG9B gene that causes neurodevelopmental clinical findings. Using CRISPR gene editing technology, we have generated knockout and knockin mouse models to study the pathophysiology of this disease *in vivo* and gain insights on physiologic functions of ATG9B. Here we present our work on these mouse models, and their genetic and phenotypic characterization.

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Keywords: autophagy, ATG9B, CRISPR, knockout, knockin, transgenic mouse, rare disease